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Contents

The Pathogenesis of Acute Renal Failure Associated with Traumatic and Toxic Injury. Renal Ischemia, Nephrotoxic Damage and the Ischemuric Episode. JEAN OLIVER, MURIEL MACDOWELL, AND ANN TRACY 1305

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THE EDITORS

CONTENTS

THE NATURE AND SCOPE OF THE PROBLEM . . . 1307

METHODS . . . 1309

MATERIAL . . . 1310

THE STRUCTURAL CHARACTERISTICS OF THE LESION IN THE HUMAN KIDNEY IN ACUTE RENAL FAILURE ASSOCIATED WITH TRAUMATIC AND TOXIC INJURIES . . . 1311

The essential tubular lesion—tubular disruption or tubulorhexis . . . 1311

The localization of the lesion of tubulorhexis . . . 1312

Nephrotoxic tubular damage . . . 1313

The collecting tubules and cast formation . . . 1319

Pigment deposition in intratubular debris and casts . . . 1319

Reparative processes in the kidney of Acute Renal Failure . . . 1321

Summary of the structural alterations in human kidneys in Acute Renal Failure . . . 1326

THE REPRODUCTION OF THE LESIONS OF THE KIDNEY OF SHOCK BY EXPERIMENTAL PROCEDURES . . . 1327

The effect on the kidney of direct ischemia produced by clamping the renal artery . . . 1328

The effect on the kidney of ischemia produced by renal vasoconstriction following trauma . . . 1331

1. Minimum functional and structural damage (K33) . . . 1332

2. Severe functional and structural damage (K32) . . . 1332

The heme pigment metabolism in the kidney of shock . . . 1333

AN EXPERIMENTAL EXAMINATION OF THE RELATION OF TOXIC AND ISCHEMIC DAMAGE IN THE KIDNEY OF ACUTE RENAL FAILURE . . . 1336

SUMMARY, REITERATION AND SPECULATION . . . 1341

CONCLUSIONS . . . 1349

THE PATHOGENESIS OF ACUTE RENAL FAILURE ASSOCIATED WITH TRAUMATIC AND TOXIC INJURY. RENAL ISCHEMIA, NEPHROTOXIC DAMAGE AND THE ISCHEMURIC EPISODE [1]

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THE NATURE AND SCOPE OF THE PROBLEM

Shortly after the publication in 1941 of his study of the "crush kidney," the late John Shaw Dunn sent tissue showing the peculiar tubular lesion that characterizes this condition to the senior author for microdissection. Somewhat later similar material was received from Dr. J. Edgar Morison of Belfast and this was followed by the gift of a considerable collection of examples of that larger group of renal lesions which was later to become known as "lower nephron nephrosis" from Dr. Baldwin Lucké.

Since that time a continuing study of these perplexing forms of renal damage has been in progress in this laboratory as confreres too numerous to thank here in person have sent kidneys both from their clinical cases and experimental investigations. Among the latter was a series of dog kidneys from Drs. Donald Van Slyke, R. A. Phillips and their co-workers in which the functional lesion of the "kidney of shock" had been reproduced and studied in great detail by clearance methods.

The result of these ten years of work in an ever-widening area of investigation had been chiefly the accumulation of a bewildering cloud of apparently unrelated minutiae in which little evidence of a pattern of unity of pathogenesis or functional correlation could be discerned. As is not infrequent in such situations, through no virtue of the present investigators other than that they persisted in their task of accumulating curiously damaged nephrons while awaiting illumination, the cloud was quite suddenly dissipated by the appearance of two related studies in renal pathology.

The first of these came to us through the privilege of a study of Dr. Homer Smith's manuscript of Chapter XXIV (Acute Renal Failure Related to Toxic and Traumatic Injuries) from his monograph, The Kidney (1). This extended and profound analysis of the functional disturbances in the group we had been studying from the structural viewpoint allowed us by a sort of inductive reversal of the causal line to proceed back from functional "effects" to a confirmation of what we had hitherto dimly glimpsed as hypothetical structural "cause," namely a disturbance in renal blood flow. The successful conclusion of the present work may therefore be looked upon as derivative of his effort.

The suspicions of the morphologists in regard to the part that renal ischemia plays in the pathogenesis of the renal lesion, even when reinforced by the considerable support of the functionalist, might well have remained, as conclusions drawn from the static data of structural appearances so often do, in the realm of reasonable hypothesis, except for the discovery by Schlegel (2) of a method for visualizing the pattern of the renal circulation by the intravenous injection of a fluorescent dye into the living animal. As will be seen in our later consideration of the problem, this procedure made possible certain crucial experiments in which the pattern of the renal circulation could be correlated with various sorts of tubular damage and so confirmed the essential unity of our pathogenetic theory.

Our investigation began therefore with an examination of the structural status of the "crush kidney" and examples of "lower nephron nephrosis" and extended to cover all "Acute Renal Failure Related to Toxic and Traumatic Injuries." Our exposition will follow a similar course, not only from a desire to show the chronological pat-

[1] This work was supported by grants-in-aid from the Life Insurance Medical Research Fund, The American Heart Association, and the New York Heart Association.

tern of our investigation but because, as will become apparent, the "crush kidney" furnished the key to the solution of the pathogenetic puzzle of the broader group.

No detailed review of the lengthy literature which concerns that form of renal damage which not infrequently follows the syndrome of shock need be restated, for the summaries, from the pathological side by Lucké (3) and Mallory (4); on its functional and experimental aspects by Van Slyke (5) and by Corcoran and Page (6); and more recently, on its clinical features by Burch and Ray (7), leave little to add. It is clear from these resumés that a most important syndrome characterized by grave renal damage and failure has been established, yet in a detailed study of them, difficulties of interpretation and correlation become so apparent as to leave the subject in the minds of most who are practically concerned with it, either as pathologist or clinician, in considerable confusion.

To set the problem clearly for our consideration it might be useful to summarize some of the difficulties which concern the pathogenesis of the renal lesions of this group and the correlation of its clinical and functional aspects with current descriptions of the structural changes that occur in the kidney.

As important in the pathogenesis of the tubular lesions the following factors are usually mentioned; (*1*) the presence of blood or muscle pigments in the tubules, (*2*) the hypothetical action of unknown "toxic products" derived from crushed tissues, and (*3*) the effects of local renal ischemia which is a reaction to the general circulatory disturbance of shock. To account for the functional disturbances, oliguria, anuria, and uremia, attention is usually called to (*1*) various sorts of structural, or assumed "functional," damage in the tubule walls, that might be reasonably supposed to allow back diffusion, (*2*) the presence of casts which might interfere with the flow of fluid through the tubules, and (*3*) the renal ischemia which might directly be the cause of decreased filtration.

To correlate with and to serve as an explanation of these pathogenetic and functional problems stands the current description of the pathological lesion in the kidney. This, as detailed by Lucké (3), is based on the original description by Dunn, Gillespie, and Niven (8) of a peculiar lesion in the distal tubule. Lucké holds that this lesion is "unique and regularly attended by characteristic functional disturbances" and that the "location of the lesions is so characteristic and unique that the term 'lower nephron nephrosis' seems more descriptive . . . (than) 'haemoglobinuric nephrosis'" ([3], p. 371). There are "relatively slight or no structural changes in the upper parts of the nephrons . . . renal corpuscles, proximal and intermediate segments. It is clear that the damage in this *disease*" (our italics) "is essentially confined to a particular component of the nephron. The identification of the several components is not difficult;" . . . "*Proximal segment.* The appearance of the lining cells of the proximal segment is often quite normal" . . . they may "exhibit cloudy swelling. Occasional fatty deposits are disclosed . . . frank degeneration or actual necrosis is very rare. *Intermediate segment.* Definite changes can seldom be found in the intermediate segment; there is neither obvious cellular injury nor precipitation of haem compounds. *Lower segment.* It is widely agreed the lower segment is damaged selectively" (p. 386) and a description of the tubular lesions of Dunn, Gillespie and Niven follows.[2]

If one returns from this description of the structural status of the damaged kidney to questions of pathogenesis and functional correlation difficulties become apparent. The first of these is: what, even in the realm of hypothesis, could be considered a likely cause of such a unique and selective damage to a limited portion of a nephron? Certainly not renal ischemia which from our knowledge of the inter-tubular circulation would of necessity involve any or all parts of nephrons indiscriminately. Renal toxic agents act on the tubule epithelium because of its functional characteristics; mercury for example is absorbed, or on the remote chance secreted, by the cells of the proximal convolution and so they suf-

[2] One finds in the literature some variation in opinion as to the localization of tubular damage; some investigators mention changes in the proximal convolution, others deny them. It would seem that these discrepancies arise from the use of a method of observation, the histological section, which is inadequate for the determination of the desired information, and since a method is at hand which can objectively settle the matter, they therefore need not be critically discussed.

fer. There is no evidence with which we are familiar that suggests the existence of analogous functional mechanisms which could produce a specifically localized damage in ascending limbs and distal convolutions.

If heme pigments are responsible for the extreme damage to tubule walls, why are these substances found so innocuous in experimental procedures? Moreover, it is an established fact that these pigments are specifically absorbed by the proximal convolution (9) and yet this segment of the tubule is described as uninjured.

Can the damage in the distal convolution be due to the mechanical effects of the blockage by pigment casts or crystals? This is a factor that at least shows the desired specificity of location in the "lower nephron," for it is here that deposits and casts occur, but their presence in many other renal conditions in which no such localization of tubule damage is found makes it difficult to believe that they can be a significant factor in the production of the "specific" tubular disruption found in the kidney of shock.

Correlation of the clinical and functional disturbances of the syndrome with the uniquely characteristic lesion described for "lower nephron nephrosis" suffers similar difficulties of comprehension. Presumably tubule fluid might diffuse or leak from any part of the renal tubular system from glomerulus to the ducts of Bellini, but the proximal tubule has been shown to be responsible for 80 per cent of water absorption (10) and one wonders if it may not be involved in a syndrome which is characterized by oliguria or anuria. It might also be expected that an uniquely localized lesion would produce a recognizably characteristic functional disturbance. It is of interest in this regard that such a functional syndrome has been described (11) and even designated as "pure" lower nephron nephrosis, but this renal disturbance bears no relation to what occurs in the kidney of shock. The aetiological factor, renal ischemia, for which strong clinical evidence exists, though it might explain at least the early oliguria could not, as we have said, on any known anatomical basis account for a lesion limited to the "lower nephron." In fact it is regularly reported that experimental renal ischemia damages particularly the proximal convolution.

All the critical reviews of these matters emphasize these confusing complications and contradictions and end with an expression of the hope that more investigation, particularly along experimental lines, may lead to clarification. The assumption is apparently made, therefore, that the problem has been correctly stated in its structural and functional aspects and that only elaboration of detail is needed for elucidation. But before more time is spent in the search for hypothetical toxic materials acting on a functionally obscure portion of the nephron by mechanisms as yet unknown it might be well to examine again more closely the relevancy of the fundamental question, for in the manner of its asking may well lie the source of our difficulties: can the status of the abnormal kidney in this syndrome be properly considered a renal disease that is characterized by an unique and specifically localized lesion in the nephron, which may be accurately described as "lower nephron nephrosis"?

In such situations a change in point of view is often helpful and that is the purpose of the present report. It details the results of the application of methods, designed to allow an inspection of the damaged nephrons in their entirety and continuity, to the study of the structural lesions in the kidney. It is obvious that such observations will be especially pertinent in a problem where specific localization of lesions is suspected.

METHODS

Though the mounting and staining of dissected nephrons has been a routine technique in this laboratory for many years, no publication, except for a general description of its use (12), has given the detail of the procedure.

Small blocks, 0.5 to 1.0 cm. in thickness, of formalin-fixed kidney are macerated in concentrated HCl at room temperature until they are quite soft. Depending on the amount of fibrous tissue in the specimen this will require from 12 to 24 hours. The macerated tissue is repeatedly washed by decanting in tap water to remove the acid and the nephrons are dissected while floating in water in a Stender dish under a binocular microscope. Individual nephrons are disentangled with needles, drawn up and arranged on a slide in the same manner as is employed with frozen sections and the excess water removed from around the specimen by blotting. A streak of vaseline is placed near each edge of the slide and a cover glass lowered upon it to form a cell which is open at each end; staining reagents may then be introduced by capillarity.

We have found the most generally useful stain to be

iron-haemotoxylin. Ten per cent Fe_2Cl_3 is drawn through the cell with filter paper and allowed to stand for three to five minutes. It is then replaced by a 1 per cent aqueous solution of haemotoxylin for five to ten minutes. The densely black nephron is then differentiated under the microscope with a weak solution of Fe_2Cl_3. The degree of decoloration may be varied to suit the purpose of the staining and final repeated flushing with tap water completes the procedure. Microscopic examination of the uncleared nephron is made in water as we have found that any attempt to clear or mount the specimens in the usual media shrinks and distorts them. In a similar manner other stains may be used: fat is well shown by Quenzel's cadmium-Sudan III mixture and aqueous toluidine blue is useful for metachromatic materials. Histochemical methods such as the common reactions for Fe may also be done on the intact nephron.

As the specimens are mounted in water the stain is not stable but fades in a few days. A special method of microphotography has therefore been devised to record permanently our observations. As a magnification of from 100 to 200 times is necessary to show cellular detail and the tubule or nephron to be photographed may be from 1.0 to 1.5 cm. in length, serial overlapping exposures on $3\frac{1}{4} \times 4\frac{1}{4}$ Wratten M plates are made as the specimen is moved across the microscopic field. Due to irregularities in focus and lack of optical flatness of field only the central third of each print is used in preparing the montage which shows the continuous structure. The overlap is therefore sufficient to allow adjoining prints to be cut on the bias and the direction and course of the tubule in its final mounted form can thus be directed to fit the form of a conventional illustrative plate with no loss of continuity. The original plates measure 18×23 inches but appear as reproduced for publication in a reduced magnification of 40 to $80\times$. Considerable sacrifice of the finer detail has occurred in this economically necessary procedure.

A nephron well stained with iron haemotoxylin from tissue immediately fixed in 10 per cent formalin shows a remarkable amount of cellular detail in spite of the preliminary treatment with concentrated HCl and the fact that it must be examined in a relatively thick (40 to 60 μ) uncleared tubule. The appearance of the various portions of the normal nephron are shown in Plates 1 and 2. In summary, the nuclei appear as unstained vesicles due apparently to the extraction of their nucleic acid. It is interesting that the hyperchromatic nuclei of regenerating cells often stain heavily and show considerable detail of their chromatic structures, both chromosomes and nucleoli (Plates 16 and 25). Whether this contrast in the effect of extraction by HCl is due to quantitative or qualitative differences might be worthy of investigation.

The most characteristic cytoplasmic elements of the renal cell, the mitochondrial rodlets, persist though their form is changed from the long, filamentous structures that are seen in well-fixed histological sections to short, elongated granules which still maintain their original linear orientation. As it is necessary to examine them through the entire thickness of the tubule, their detail is somewhat confused but since mitochondrial elements are usually considered to be especially susceptible to the action of acetic acid, it is surprising to see how well they are preserved after the treatment with concentrated HCl (Plates 2 and 28). Other cytoplasmic structures, such as the brush border are clearly shown (Plate 2C) and the basement membrane of the tubule stands out as a sharply defined, homogeneous, thin band that outlines the lateral aspects of the tubule (Plate 2A).

The examination of nephrons from routine human autopsies suffers the same but no more difficulties in cytological examination and interpretation than are encountered in preparations of this material by the technique of the histological section. With both methods, the mitochondrial pattern suffers from the unavoidable delay in fixation of the tissues. It has been found by experiment that 15 minutes at room temperature is sufficient to alter considerably the cytological detail, so that the most common appearance noted in the renal epithelium of normal dissected nephrons of human origin is that of a darkly stained cytoplasm filled with irregularly distributed fine "granules," and, as in histological sections, the brush border may be obscure. As will appear in our descriptions of the damaged nephrons, various cellular abnormalities, cloudy swelling, hyaline droplets, fat accumulation, vacuolar hydropic degeneration, pigment deposit, regeneration with the production of an atypical epithelium, cast formation, and all the other phenomena of renal tubule alteration and damage are seen in an analogous but very different aspect from what is apparent in the conventional pattern of the histological section. It is perhaps unnecessary to add that the latter method was used in all cases to supplement our study of the dissected nephrons.

MATERIAL

The material of this study is of three sorts; first, human kidneys from a large variety of examples of renal damage associated with the syndrome of "shock" and other forms of acute renal failure (Table I); secondly,

TABLE I

Clinical aetiology of 54 fatal cases of Acute Renal Failure

Crushing injury (bombing)		7
Burns		7
Transfusion accidents		5
Malarial blackwater fever		5
Obstetrical complications (Eclampsia, hemmorhage, placental separation)		5
Surgical shock (Perforated gastric ulcer)		1
Cold paroxysmal hemoglobinuria		1
Poisoning		23
Sulfonamides	10	
Mercuric chloride	7	
Di-ethylene glycol	2	
Carbon tetrachloride	2	
Potassium chlorate	1	
Mushroom poisoning	1	
		54

dog kidneys from a series of experiments in which the pathogenetic factors and functional disturbances following induced shock had been examined; and thirdly, rabbit kidneys from experiments designed to show the actiological relations of the various factors involved in renal damage due to toxic injury. We shall present first the essential lesions that were found in the human kidneys and then endeavor to show how these lesions may be interpreted in the light of what was found under analogous experimental conditions.

THE STRUCTURAL CHARACTERISTICS OF THE LESION IN THE HUMAN KIDNEY IN ACUTE RENAL FAILURE ASSOCIATED WITH TRAUMATIC AND TOXIC INJURIES

Since the general nature of the clinical and pathological syndrome has been thoroughly examined and described by many investigators and these studies well summarized by Lucké (3) and others we have omitted much of our detailed case record description, both clinical and pathological, except where it was pertinent to some particular point in our exposition. This seemed permissible for little new has been added by our investigation to previous descriptions of the generality of the pathological status of the abnormal kidneys; a considerable addition results, however, from the different aspect of the lesions and from the integration of them into the general picture of renal damage that is made possible by their observation in the continuity of the complete nephrons.

There are two questions regarding the structural lesion with which we shall be especially concerned and both will be of crucial importance in the final interpretation of its pathogenetic and functional significance; first, the nature of the tubular lesions, and secondly, the distribution of these lesions not only in the individual nephron but among the nephrons generally. Subsidiary to these two problems are such matters as the nature and degree of tubular occlusion, the occurrence of interstitial tissue reaction, the presence and effect of deposits of pigment, and the reparative changes that may occur in the damaged nephrons.

The essential tubular lesion—tubular disruption or tubulorhexis

As in all examples of renal disease, a considerable variety of "tubular lesions" is present in the kidney when acute renal failure occurs as a result of traumatic or toxic injury. On this background of non-specific renal damage is found, however, a lesion peculiar to and therefore characteristic of the group, namely a localized destruction of the entire tubule wall. This lesion was first described in histological sections by Dunn and his co-workers in the crush kidney and its occurrence in other related conditions has been noted with emphasis in varying degree by all subsequent investigators.

The lesion is clearly shown in all the varied manifestations of its development in preparations of complete nephrons; as one follows the course of the intact tubule, quite suddenly a place is found where the basement membrane is broken, frayed or disintegrated and the epithelial lining disrupted and necrotic. The result is a solution of continuity which may include only a part of the wall of the tubule or which completely interrupts its course so that the fragmented remnants lie between a still intact and well preserved proximal portion and its distal prolongation (Plates 3–27). The lumen thus lies open to the intertubular interstitial tissue and its capillaries and veins (Plates 5 and 10).

The lumen of tubules so affected may or may not contain solid material in the form of debris or of a consolidated, well-formed cast. The frequency of association of rupture and cast is apparently determined by the situation of the lesion: if the former occurs in the ascending limb of Henle's loop or in the distal convolution where casts are frequent, the two are not uncommonly found together (Plate 5). That this association can, however, be little else than fortuitous is emphasized by the fact that large and extensive casts, many containing blood pigments, are frequently found in the distal convolution with no lesion in the tubule wall (Plates 7, 15, 17 and 19).

A great variation in the appearance of the disrupted tubule results not only from the extent of the damage and the presence or absence of solid material in its lumen but also from the reaction that may occur in both the tubule wall itself and in the mesenchymal tissue in the neighborhood of the rupture.

In certain instances a simple solution of continuity describes the lesion, as the tubule wall appears dispersed into fragmented debris and necrotic desquamated epithelial cells that are loosely held together by the thin, disintegrating basement membrane which still maintains in some degree

the original contours of the tubule (Plates 6, 8, 14 and 23). There is little or no reaction in the peritubular tissue. In other examples, the interstitial reaction about the ruptured segment is extreme so that in the dissected specimen tufted masses of connective tissue fibers surround the damaged tubule and are so firmly attached to it that they cannot be removed with the dissecting needles (Plates 4, 9, 11 and 26). Or the surrounding reaction is seen as definite "granulation tissue" with a proliferation of capillaries among the mesenchymal elements that not only surround the ruptured tubule but grow into its disrupted lumen (Plates 5, 10 and 25).

The reaction of the epithelial cells to damage of the tubule wall will be considered in detail in a later description of the reparative processes that occur in the nephrons. As a rule the epithelial cells are remarkably well preserved up to the very edge of the tubular disruption and by their large size and irregular shape show definite evidence of proliferation. At times an actual extension by growth of a thin, flattened epithelial margin may be observed accompanied by irregular clusters of atypical, hyperchromatic nuclei (Plates 16, 17, 19, 21, 24 and 25). However, as a result of the peculiar character of the lesion, the basement membrane being no longer present to support the proliferating epithelium, restitution of the damaged tubule wall seldom results.

An examination of the illustrations of this unusual tubular lesion will show better than many words the variations that are built up of combinations of the elements we have described. No typical combination has been found in any particular clinical variety of the syndrome, though in any one case they may tend to resemble each other.

The localization of the lesion of tubulorhexis

Except for its three-dimensional aspect, our description so far of this characteristic tubular lesion does not differ essentially from that of previous studies based on its appearance in histological sections. When the second of its supposed characteristics is considered, *i.e.*, its specific localization in the "lower nephron," a departure from current concept becomes apparent as the direct result of its examination in the continuity of complete nephrons.

In all of the kidneys which we have examined from every clinical variety of the syndrome the disruptive lesion has been found in every portion of the nephron from the origin of the proximal convolution at the glomerulus (Plate 13) to the connecting tubule where it joins the collecting ducts (Plate 4). In no particular clinical variety of kidney have we observed any especial distribution and even in a single kidney there is great variability in its location in the involved nephrons. Moreover, even in severely damaged kidneys, not all nephrons are affected and in the moderate example, only occasional ones. Randomness of distribution, both in the nephron and among nephrons, is therefore another characteristic of the disruptive lesion.

Although the disruptive lesion is found in all segments of the nephron there are two locations where it is most obvious. The first of these is the terminal portion of the proximal convolution where it dips into the medulla (Plates 3, 6, 14 and 18) and the second is the distal convolution and connecting tubule (Plates 4, 5, 7 and 10). Actual disruption and fragmentation of the tubule is somewhat less evident in the periglomerular portion of the proximal convolution although it may be found here and often is easily identified in the dissected specimens by means of the peritubular adhesions which surround it (Plates 9 and 26). Short stretches of damaged proximal tubule alternating with well preserved tubule are common enough, however, and produce a patchy irregularity in the appearance of the stained specimen that is in striking contrast to the evenly stained pattern of the normal convolution (Plate 3).

In the terminal portion of the proximal convolution the tubular damage is seen in its maximum development: the disintegrating basement membrane exists only in thin, barely perceptible outline and the epithelium is necrotic and desquamated. Long stretches of tubule may thus be denuded of their epithelial lining and as a result are reduced to a third of their original diameter (Plates 3, 6 and 14). They thus no longer have the typical appearance of proximal convolutions but resemble damaged limbs, either descending or ascending, of Henle's loop. In such instances, even in the continuity of the dissected nephron, the point of transition to the thin limb of Henle's loop with which they are continuous can be de-

termined only by the fact that proximal convolutions have a relatively constant length in each species. These terminal portions of the proximal convolution, it will be remembered, lie in the outer stripe of the outer zone of the medulla interspersed among ascending and descending limbs of Henle's loop, a topographical configuration which makes any identification of one from the other in histological section quite impossible (Plates 3 and 7).

Although debris and loose conglomerations of desquamated cells may be found in the damaged proximal convolutions, one rarely finds consolidated "casts" as the term is applied to the coagulative plugs that are common in ascending limbs, distal convolutions and collecting tubules of all forms of kidney disease (13).

As might be expected from the differing morphological and functional characteristics of the distal convolution and connecting tubule, tubular disruption in these regions produces lesions of a somewhat different appearance from those seen in the proximal convolution. The damage may involve a short segment or a considerable portion of the affected tubule. Around the point of disruption peritubular adhesions and ingrowths of reactive granulation tissue are frequent and extensive (Plates 5 and 10). A well-formed coagulative cast may or may not be present at the site of rupture; however, since cast formation is frequent in this region of the nephron, many but not all damaged distal convolutions contain such a coagulum (Plate 25). Not infrequently, however, it is found to lie above or below and not contiguous to the point of tubular rupture (Plates 4 and 19).

The disruptive lesion is frequent in both limbs of Henle's loop and may consist of a repetition of short damaged segments or of long stretches that include the greater part of the limb.

To summarize, the distribution of the disruptive lesion in a single nephron is entirely random: there may be severe damage in the proximal convolution and none in the remainder of the nephron, though in such cases the ascending limb and distal convolution are certain to contain much debris and probably casts. In another nephron from the same kidney the distal convolution may show extensive disruption, either with or without cast formation, and the other segments be normal. In another, only the ascending limb of Henle's loop is involved, either in its entirety or in segmental fashion with stretches of well preserved tubule intervening. Nor are all the nephrons affected, for even in severely damaged kidneys a considerable number contain only casts in the ascending limb or distal convolution and the tubule wall is intact throughout.

* * * * * *

The disruptive lesion is clearly quite different from the damage that results from the action of toxic agents on renal epithelium and which is commonly considered characteristic of a "nephrosis." In the toxic tubular lesion the renal epithelial cells undergo regressive change, die, desquamate and disintegrate to debris that may be seen filling the space outlined by the basement membrane. This structure suffers no visible change but remains intact and on its supporting surface regenerating epithelial cells may ultimately reconstitute the tubule. These tubular lesions, as will be seen in our next subdivision, are commonly found in certain examples of acute renal failure. Plate 16A and B[1] show the contrast between disruptive and toxic lesion.

Two sorts of tubular lesion, strikingly different in the nature of the damage that results to the tubule both in regard to structural alteration, location in the nephron and the degree of later regenerative repair that is possible, must therefore be recognized. For these reasons it would seem that they should be distinguished by appropriate terms: nephrotoxic tubular damage describes the one and some such term as tubulorhexis seems appropriate for the disruptive lesion.

Nephrotoxic tubular damage

If the clinical origin of many of the examples of "lower nephron nephrosis" is considered, it would seem certain that toxic damage to the kidney must be a factor in the development of whatever renal lesion may be present. Such, for example, are the 20 cases of poisoning in Lucké's summary which includes such toxic agents as mushrooms, denatured alcohol, arsenicals, carbon tetrachloride, and phenol.

As we have mentioned in our discussion of the difficulties involved in the concept of "lower nephron nephrosis," the presence of these cases in the syndrome, as its structural status has been de-

scribed, is a source of considerable perplexity, for all past experimental study of toxic substances indicates that they damage not distal but proximal convolutions (14). Our findings to this point might seem on first thought to have at least cleared this difficulty for we have found that the proximal convolution is as badly damaged in some fashion or other as is the distal in even the classical example of the "crush kidney."

And so it might be thought that at this point in our exposition a major stroke of simplification would be possible: under the general heading of Acute Renal Failure we have only to put the cases of renal poisoning in one group and the kidneys of "pure" shock in another. The first could be the plain or garden variety of "nephrosis," its lesion being that of nephrotoxic damage and the latter, if not "lower nephron," at least some other special brand characterized by the presence of tubulorhextic lesions.

But any such procedure would be to cut, not unravel the pathogenetic tangle for as our dissections will show we are faced with the fact that the examples of renal poisoning have both sorts of tubule lesion, tubulorhextic and nephrotoxic. Lucké was therefore well justified in recognizing common qualities in kidneys from crush injury and carbon tetrachloride poisoning. Moreover the clinical and functional evidences, which we shall consider later, strongly suggest, or one might say demand, a certain degree of unity in the overall group of Acute Renal Failure.

The contrast between the structural changes of tubular damage due to a nephrotoxic agent and those of the tubulorhextic lesion, therefore, still remains as a complication to pathogenetic theory. Its solution requires experimental examination and will therefore be delayed for later consideration. At this time we shall describe typical examples of human kidneys that showed in significant degree nephrotoxic tubular lesions as well as the disruptive tubulorhextic damage.

Again to contrast briefly the two types of tubular damage, the most distinctive difference is found in the lack of the element of disruption of the tubule wall in nephrotoxic damage: the epithelial cells are dead and may ultimately be reduced to granular debris that packs, sausage-like, an intact basement membrane (Plate 16A). The location of the toxic lesions also shows a striking difference from what is observed of tubulorhextic disruption: the former occur only in the proximal convolutions and, moreover, are evenly distributed among all the nephrons.

Our material consisted of 21 human kidneys from instances of definite clinical poisoning. These were supplemented by the kidneys of 29 experimental animals, dogs and rabbits, which were used in our study of the relation of renal ischemia to toxic renal injury which will be described later. In detail, the examples of human kidneys dissected were as follows:

Mercuric chloride	7
Potassium chlorate	1
Di-ethylene glycol	2
Mushroom	1
Carbon tetrachloride	2
Sulfonamides	10

It might be objected that kidneys damaged by mercuric chloride should not be included in our list, for although the other forms of renal poisoning that we have given are accepted as proper examples of lower nephron nephrosis, it is generally admitted by those who have supported this concept that corrosive sublimate is the classical example of a toxic agent which damages the "upper" nephron, or, in more accurate terms, the proximal convolution. As we have stated, this fact has been experimentally established (14, 15). From what we have seen in our examination of the "crush kidney," the example *par excellence* of the "lower" nephron nephroses, the acceptance of the "unique" characteristic that presumably establishes this latter syndrome is subject to considerable reservation, for the tubulorhextic lesions were found as frequently in the proximal as in the distal portions of the nephrons. We shall now see that the lesion in the "sublimate kidney" is also less simple than the classical description indicates when it is viewed in the continuity of dissected nephrons from fatal cases of human poisoning. To the resolution of this added complication we shall return at a later time; at present we shall describe without theoretical comment what was found in the kidneys of those who had died of the acute renal failure associated with toxic injury.

A 44 year old woman took six mercuric bichloride tablets (3.0 gm.) and entered the hospital with nausea and abdominal cramps three hours later. Her blood

pressure was 86/60. Five hours after the ingestion of the poison, BAL treatment was begun and she was given 2 cc. every four hours for six days, and then 1 cc. every 12 hours for five days. During the first 42 hours the patient had diarrhea, vomited and suffered abdominal cramps. Fifty cc. of urine were passed in the first 24 hours, then none for five days. The urinary output rose slowly for six days to 935 cc. and then fell to an average of 120 cc. until death. The NPN on the second day was 53 mg. per cent rising to 450 on the 28th day. On the 29th day she died. Clearances of inulin, creatinine and PAH were infinitesimal on six occasions.

Histological sections showed extreme tubular damage concerning the location of which we need not speculate until our dissections, which accurately determine the seat and nature of the lesions, are described. A most striking histological feature was the interstitial edema and infiltration with monocytes and leucocytes which had occurred throughout the cortex and outer medulla. This reaction was especially intense and at times could be seen to originate around disrupted cross sections of tubules which had the general location of the distal convolution. Extensive regeneration as evidenced by atypical epithelial cells either lining the tubule wall or forming irregular masses that impinged on the tubular lumen could be seen in the sections (Figure 1).

In the dissected nephrons extensive tubular damage of the nephrotoxic type was present in

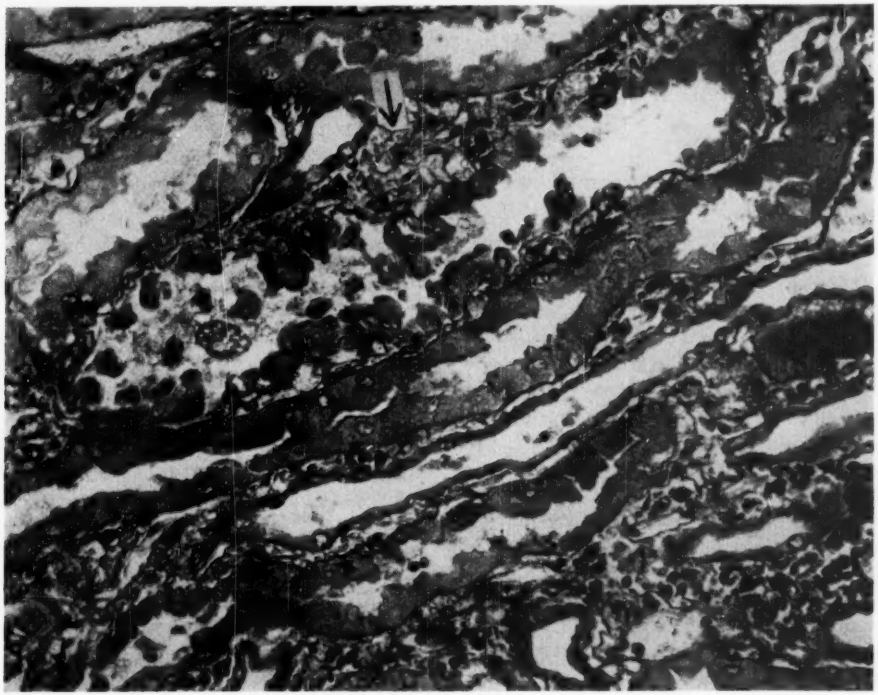


FIG. 1. SUBLIMATE POISONING—SECTION THROUGH TERMINAL SEGMENTS OF DAMAGED PROXIMAL CONVOLUTIONS

The normal diameter of these segments is represented by the two lower tubules, the epithelium of which is largely necrotic. Above is a section through a greatly swollen tubule apparently similar to the disruptive lesion shown in Plate 16B[3]. Note the heaped up irregular mass of proliferating epithelium and at the arrow, the disruption of the basement membrane. Below to the right the inflammatory infiltration in the interstitial tissue is apparent. Magnification 250 ×.

the proximal convolutions: scattered along the course of these damaged convolutions were stretches of disruptive tubulorhexis in which the tubule was greatly swollen, its basement membrane destroyed and the lumen open to the exterior. Plate 16 shows such a lesion in the terminal portion of a proximal convolution; to the upper left and horizontally, the necrotic but still intact tubule can be seen, then follows, on the extreme right, a stretch of disrupted tubule and the convolution ends in the narrowing descending limb, again intact as a tubule but necrotic. The dissection of such nephrons was exceedingly difficult since their continuity had been practically destroyed by the disruptive lesions. In these lesions, masses of proliferating cells could be seen with well stained prominent nuclei. As we shall at a later point return to the reparative processes that occur in damaged kidneys they are only noted here in passing.

Plate 17 shows a collecting tubule (left) and two attached distal convolutions. In the greater part of all, the lumen is filled with a solid cast which stains a deep black with the iron haemotoxylin. In sections, similar casts stained with eosine and gave no reaction for heme pigments or iron; they are therefore simple hyaline or granular casts composed of coagulated protein and cellular debris. The detail in the lowermost of the distal convolutions is largely obscured by this cast formation. The first portion of the uppermost distal convolution has been almost completely relined by a thin layer of atypical epithelial cells; at certain points the disruptions are not completely closed. Further evidence of the incompleteness of tubular restitution is seen in the irregular strictures and dilatations of what was originally a smoothly contoured tubule.

Our five other examples of fatal poisoning with mercuric chloride added little else to the picture of renal damage by this agent. The survival periods were four, eight, 11 and 14 days and one unknown, so that there were individual differences especially in regard to regeneration in the various cases. Of the six, four showed interstitial edema and leucocytic and round cell infiltration to a considerable degree and one only focally around ruptured tubules. The remaining case showed, in conformity with the classical description, no evidence of an inflammatory process. In only one case was there calcification of luminal debris.

Another example of the renal lesion associated with toxic injury is the case of a man of 19 admitted for nausea, vomiting, abdominal cramps and black urine of 30 hours duration. Before death he admitted to eating two tubes of tooth paste containing 40 per cent of potassium chlorate. The coffee colored urine showed 4+ albumin, on admission. The patient was given infusions of 5 per cent glucose and sucrose; vomiting continued to the end. The NPN on the fourth day was 128 mg. per cent; on the ninth day, 165. On the fifth day the urine volume was 80 cc. in 24 hours and then rose to 200 cc., finally to decrease with death on the 11th day.

Sections of the kidney showed extensive damage to the tubular epithelium that will be described as it appears in our dissected nephrons; the glomeruli showed no lesion other than evidence of increased permeability to plasma proteins (Figure 2). In the sub-cortical medullary zone, that is, in the region occupied by the terminal segments of the proximal convolution, there was a focal infiltration with round cells, plasma cells and occasional polymorphonuclear leucocytes. As our dissection will show, it was in these terminal segments that the greatest damage had occurred.

Plate 18 is a typical complete proximal convolution. Its first half shows a relatively well preserved although swollen tubule but there is little evidence of a nuclear pattern. The terminal portion down to the origin of the narrow limb of Henle's loop is severely damaged with necrosis, desquamation and disintegration of the tubular epithelium. Nuclear debris and cellular detritus fill the course of the tubule. Other proximal convolutions showed disruptive lesions.

In Plate 19 is shown an example of an ascending limb and distal convolution to its junction with a collecting tubule. The nuclear pattern of the swollen ascending limb can still be recognized. In the distal convolution, horizontally center, is a greatly swollen area of tubular disruption. The basement membrane has disintegrated and proliferating epithelial sheets grow at random with no reconstitution of the tubule wall. A dense cast follows and then another area of disruption.

Our two cases of poisoning with di-ethylene glycol will be but briefly mentioned here as they are to be considered in detail at a later point when the reparative processes that occur in the kidney

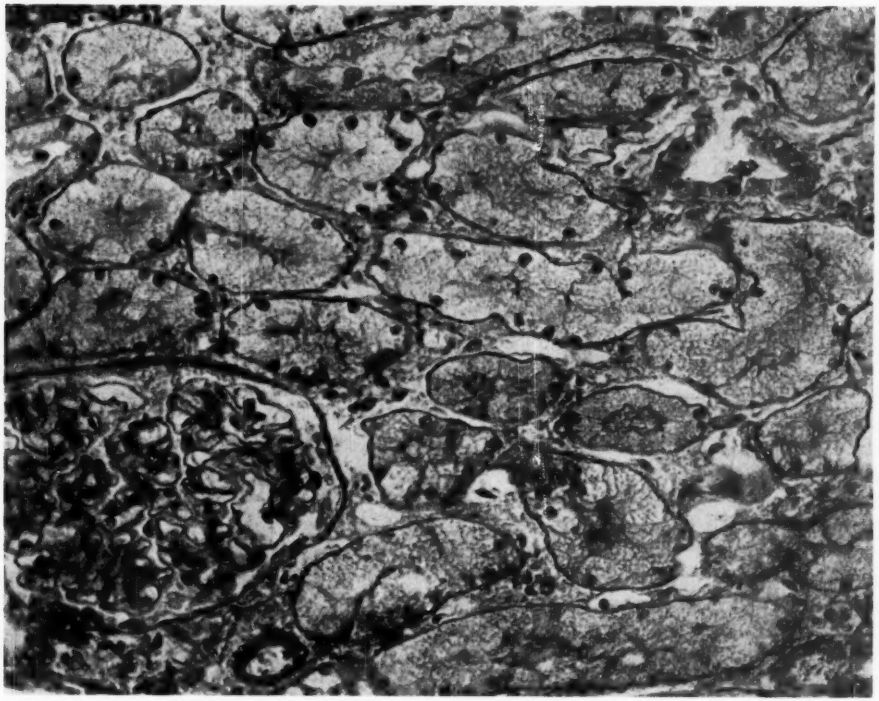


FIG. 2. POTASSIUM CHLORATE POISONING—SECTION THROUGH THE PERIGLOMERULAR LOOPS OF A PROXIMAL CONVOLUTION

Some nuclei persist in the swollen vacuolated epithelium but in many, necrosis is complete. The cross sections of this figure correspond to the first half of dissected specimen of Plate 18. Magnification 250 ×.

of acute renal failure are described. Sections of the kidneys of two individuals who took the toxic agent at the same time showed on the 12th day the typical vacuolar nephrotoxic lesion in the proximal convolutions. In sections of the kidney from the individual who had survived for three weeks, areas of cellular infiltration with monocytes, plasma cells and occasional polymorphonuclear leucocytes were present in the sub-cortical zone and in foci throughout the cortex. In the dissected specimens there was extensive nephrotoxic damage in the proximal convolutions as well as disruption and dissolution of the basement membrane (Plate 22). In the ascending limbs disruptive lesions were also frequent and more extensive (Plate 23).

One case of mushroom poisoning showed in its proximal convolutions a necrosis of the epithelial cells of its terminal medullary portion as well as frequent tubulorhextic disruptive lesions scattered along the course of the entire nephron from proximal to distal convolution. The same description might be applied to the lesions noted in the nephrons of a case of carbon tetrachloride poisoning. In both cases, focal areas of monocytic infiltration were present in the sub-cortical medulla in the region of tubular damage.

Ten examples of the damage done to the nephron by sulfonamides were examined. The treatment of the tissues with concentrated HCl had removed all traces of crystalline deposits in our dissected specimens. As has been noted in the literature, considerable variation was seen in different cases in the detail of the renal lesions.

All showed in histological sections an inflammatory interstitial reaction, either diffusely in the cortex or sub-cortical medulla or in a granulomatous aspect around ruptured tubules. It was the rare example which showed even in histological section the presence of crystals. No special means had been taken in the preparation of this material to preserve these structures, but it seems unlikely that they could have been present in every ruptured tubule, and several of the few that were found lay in tubule cross sections which showed no damage. With no very positive evidence in the matter, but with the knowledge that similar disruptions must have another origin in the other forms of renal toxic damage, we are therefore somewhat sceptical towards the suggestion that rupture of tubules in sulfonamide poisoning is the result of perforation of the wall by crystals.

As has also been the experience of others, we have found considerable variation in the types of damage to the tubule of the nephron in our dissected specimens. In all ten cases definite disruptive lesions were found throughout their entire length from proximal convolution, especially in its terminal medullary segment, to the connecting tubule. In four of the ten cases there was frank necrosis of the middle and terminal portions of the proximal convolution. In Plate 20 is shown a complete example: the tubule is swollen and the nuclear pattern is obscure throughout. In several stretches the disintegration of the tubular epithelium is evident and in its terminal portion it is frankly necrotic.

In the other six cases the nephrotoxic lesion in the proximal convolution was, if present, so slight as to be uncertain. As we have mentioned in the description of our methods and as will be apparent in the discussion of our results, human autopsy material is unsuited even under the best of conditions for the determination of slight epithelial alterations, for both in histological section and dissected specimen the demonstration of the finer cytoplasmic detail, such as the condition of the mitochondrial rodlets, requires immediate fixation.

In Plate 21 an ascending limb, left lower, with a well preserved nuclear pattern passes up to a distal convolution in which there are several disruptive lesions. An attempt at regenerative repair may be noted but the break in the tubule wall is not closed. Another distal convolution joins from above and the collecting tubule, arching downward and then up to the right, is filled with irregular masses of detritus and debris, but its cellular pattern is perfectly preserved.

* * * * * *

Later in our exposition we shall return to a further consideration of the damage that is found in kidneys of acute renal failure associated with toxic injury, first when the problem of repair in the damaged kidney is discussed and later when an experimental examination of the problem of the aetiological relations of the two sorts of tubular lesion, nephrotoxic and tubulorhextic, is examined. Considerably more data will thereby be available concerning renal toxic injury.

For the present, two points may be emphasized. First, all examples of fatal acute renal failure due to poisoning that we have examined have shown not only the classical nephrotoxic necrotizing lesion in the proximal convolution but also, scattered throughout the length of the nephron, the disruptive tubulorhextic lesion that was the prevalent feature in the kidney of crush injury.

The second is the regular occurrence of an interstitial inflammatory reaction in greater or less degree in fatal acute renal failure whether it had arisen on the clinical basis of traumatic or toxic injury. This reaction was particularly prominent in the sub-cortical medulla where the terminal portions of the proximal convolution lie which, as our dissections have shown, are the frequent sites of disruptive tubular damage: it was also prominent in histological sections in the cortex around disrupted distal tubules.

These interstitial reactions have been generally recognized as an integral aspect of the renal lesion in certain of the "lower nephron nephroses," such as the crush and sulfonamide kidney, but at least not emphasized or even mentioned in other members of the group of frank toxic origin. This perhaps stems from the subconscious feeling that the essential toxic lesion, following sublimate for example, must be "non-inflammatory," a fact firmly established indeed by extensive experimental evidence. We see again, therefore, the semantic, yet nonetheless real confusion that has resulted from the introduction of the term "nephrosis" and its variant of the "lower nephron"

into the nomenclature of renal disease. It is not surprising that A. and H. Bergstrand object most strongly to the "-osis," and insist quite logically that it must be an "-itis" (16), for originally (17), and to many authorities today, this is the distinction for which the etymological curiosity was coined. Since, as we have said elsewhere, "nephrosis" in its protean connotations is "all things to all men," it is doubtless futile to insist on any particular meaning in the use of the term. Leaving the solution of the semantic aspect of the problem to the ages, we can at least offer an explanation of the apparently incongruous fact that the damage of fatal acute renal failure due to a toxic injury that is characterized by the necrotizing, non-inflammatory tubular lesion also shows an inflammatory element in the interstitial tissue of the kidney. The latter is obviously not the result of the toxic action which caused necrosis of the epithelium of the proximal convolution but, as can be seen from its localization, is related to the disruptive tubulorhextic lesion. The "cause" of the interstitial reaction is therefore tied to the aetiological factors that operate in the production of tubular disruption and these will be examined by experimental methods at a later time.

The collecting tubules and cast formation

No damage has been found in the collecting tubules of any of the kidneys of Acute Renal Failure save compression of epithelial cells and occasional desquamation; these ducts, however, contain casts and debris in proportion to the amount of damage that has occurred in their contiguous nephrons. These occlusions are essentially similar in their morphological aspects to those seen in all forms of renal damage (13), but a description of them as they occur in the kidney of Acute Renal Failure may be repeated, as the appearance of the tubular "cast" is strikingly different in dissected material from what is seen in the conventional histological section. Functional implications drawn, as they usually are, from the latter are apt to be inadequate. Some casts, when seen in their entirety lying *in situ* in the upper or middle branchings of the collecting system, are relatively short plugs and their appearance is not very different from what is observed in longitudinal histological sections of a collecting tubule (Plates 17, 21 and 25). Many other coagula are of relatively enormous extent: they may be traced as a continuous structure in dissected specimens from their origin in the ascending limb of a nephron, through its distal and connecting tubule into the peripheral branch of the collecting tubule and then down through the cortex to a point deep in the medulla. In its course through the cortex are found branch-like extensions of solid material continuous from other damaged nephrons which drained into the occluded collecting duct, so that the whole coagulum forms a hydra-like be-tentacled structure 2 to 3 cm. in length (Plates 4 and 17). Yet in a histological section such a complex object might appear as a few scattered sections of "tubules and casts" that bear no apparent topographical relation to each other.

These ramifying coagula are not homogeneous in structure throughout their considerable length. In some, long stretches are composed of clear hyaline material and in this substance are irregular collections of granular deposit either diffusely scattered or conglomerated in irregular masses. The dark staining granular material is evidently in part cellular debris, for fragments of epithelial cells are at times recognizable (Plate 23). Pigments and crystals, to be described more fully later, are also present. In other examples the entire extent of the coagulum may be opaque and granular from an admixture of heterogeneous debris.

Pigment deposition in intratubular debris and casts

In considering the appearance of the heme pigments in our preparations it should be recalled that the kidney tissues had been macerated in concentrated HCl for 24 to 48 hours preliminary to dissection and staining of the nephrons. This treatment might be expected to alter both the physical and chemical nature of blood pigments but, as will be apparent, our findings in the dissected material agree in all essentials with previous observations made on fixed histological material.

As has been the experience of other investigators, we have found heme pigment in all of the kidneys that we have examined from cases where myoglobin or haemoglobin was liberated into the blood stream as part of the primary cause of the clinical disturbance. In these and many other instances blood transfusions had been given and though a

transfusion reaction may not have been noted in the clinical record an added source of the products of blood degradation was therefore present. In the cases in which there was no evidence in the clinical record that haemolysis or muscle injury had occurred the typical disruptive tubular lesions were found with no histochemical evidence, such as the benzidine or Prussian Blue test, of heme pigment in casts or tubular debris. It is noteworthy, therefore, that in this group of constant and similar renal disturbances associated with shock and related conditions of Acute Renal Failure there is from their very origin no constancy in regard to any aetiological factor that concerns heme pigmentation.

The appearance of the pigment in the dissected nephrons differs as it is seen in fully formed casts, *i.e.*, in the upper ascending limbs of Henle's loop, the distal convolutions and the collecting tubules, or in the loose unconsolidated debris that is found in the proximal convolution.

The appearance of the heme pigments in casts as they lie *in situ* in the lumen of the dissected nephrons is essentially that which one would expect to see when viewing in their entirety the objects that have previously been described in histological section. What would otherwise be designated as a "hyaline" cast may show an evenly diffused, faint brownish discoloration in the unstained specimen. When stained with iron haemotoxylin these coagula take on a dense black color, the same reaction that is seen in them and in red blood cells in histological sections. When tested for free iron by the Berlin Blue method the casts in distal portions of the nephrons and in the collecting tubules are negative, but are positive to the benzidine reaction. The treatment with HCl has therefore not affected these histochemical reactions. In long casts that run continuously through considerable lengths of tubule it is often observed that the concentration of pigment varies greatly and at times with remarkable abruptness in various portions of the coagulum. Histological sections of such a structure would be interpreted as several separate and different sorts of casts.

In other examples the heme pigment is less evenly and finely dispersed in the substance of the matrix so that a definitely granular appearance is produced which may be exaggerated to the extent that conglomerates or sphericles of pigment and, in the extreme case, deformed crystalline deposits are seen. We have never observed crystalline deposits unassociated with organic matrix. In restricted stretches of tubule this appearance may be simulated but by following along the tubule it becomes evident that these apparently isolated "free" crystals form a part of a coagulated structure.

The general distribution and optical characteristics of the heme pigment in the casts in the isolated nephrons have therefore the appearance that might be expected if a column of protein-containing fluid with irregularly distributed concentrations of heme pigment had coagulated with the production of a varying and discontinuous pattern of pigment deposit, from a molecularly dispersed, diffuse staining to the inclusion of relatively pure crystalline masses.

As was the case in our correlation of the presence of casts with tubular disruption, no positive result was observed between the presence of pigment deposition, either free or in casts, with tubular lesions. If a cast was fortuitously present at the point of rupture, it often but not always contained heme pigment, but other casts of great extent filling continuously the ascending limb and distal convolution with extreme concentrations of granular pigments were commonly observed surrounded by an intact and well preserved tubular wall (Plates 4, 7, 10, 12, 15 and 19). There was therefore no evidence that the presence of large concentrations of heme pigment had any specific ill-effect on the tubular epithelium.

We have found little mention in previous studies of shock kidneys of the occurrence of heme pigments in the proximal convolutions. This seems remarkable for much experimental work has shown that under normal circumstances these pigments are selectively absorbed, stored and metabolized, as are other proteins (9), by the epithelial cells of this portion of the nephron (18). The physiological conditions under which heme pigments occur in the proximal convolution are therefore very different from what obtains in the distal nephron where the pigment lies in the tubule lumen unabsorbed and unchanged, as is shown by the positive benzidine and negative Berlin Blue tests, by the activity of the renal epithelium. Our findings in both sections and in dissected nephrons of human kidneys were difficult of interpretation

until we had studied these processes in the kidneys of experimental shock. Our description and discussion of heme pigments in the proximal convolution will therefore be deferred until the latter have been described.

Reparative processes in the kidney of Acute Renal Failure

Whereas the clinician has opportunities to follow the restoration of function in the damaged kidney the pathologist sees only the more extreme of renal lesions and only those that did not recover. His factual knowledge is therefore confined to what he sees of inadequate attempts at restitution of a normal structural and functional status in the damaged kidney; to this he commonly appends by a sort of imaginative extrapolation a description of what he thinks the course of adequate repair must be in those conditions of lesser damage of which he has no first-hand experience. The resulting descriptions of what is called "tubular regeneration" are perhaps adequate for concepts of renal structure based solely on the study of histological sections which describe the kidney as composed of "glomeruli" and various sorts of "tubules," but they prove quite meaningless in considering the repair of a tortuous and tenuous organ like the nephron where continuity is essential to normal function. Failure of restitution of a freely draining lumen at a single point in its course, for example, or the persistence of a single minute hole in its wall, may destroy the total functional integrity of the whole organ so far as its excretory capacity is concerned, no matter how many regenerating epithelial cells may have been seen by the observer's imaginative eye to be "creeping beneath the dead debris to reline the most damaged areas."

A consideration of the appearance of the disruptive lesion in Plates 5, 10 and 16 will show at a glance that its most severe form is probably irreparable. Disruption or dissolution of the basement membrane, the characteristic that distinguishes this lesion from that of nephrotoxic damage, is the factor which prevents repair, for not only is there no directing surface on which regenerating epithelial cells "creep" to reform the tubule, but the gap in the tubule wall is invaded by granulation tissue which fills and obliterates the tubule lumen. Here again, then, is seen the antithesis of tubular disruption and the tubular lesion of nephrotoxic damage, for in the latter, as exemplified by the kidney of the moderate example of sublimate poisoning at least in its experimental form, regenerating epithelium relines the bare but intact membrana propria so that both structure and function are restored.

It may be that minor disruptions of the tubule wall are plugged by proliferating cells; certainly evidence of epithelial regeneration, as contrasted to nephron reconstitution, is frequently found in the dissected specimens. At the margin of the tubular disruption may be seen large atypical epithelial cells with hyperchromatic nuclei, an example of exuberant but obviously futile regeneration, and analogous abortive attempts at repair, as evidenced by an irregular heaping up of newformed but misplaced cells, are shown in Plates 16, 17, 19, 21, 24 and 25.

In instances where considerable amounts of blood pigment have flowed through damaged tubules, as in the kidney of the transfusion accident, it can also be seen that the regenerating renal epithelium has regained at least certain of its functions. In Plate 12 long stretches of damaged proximal convolution have been relined with an atypical epithelium containing irregularly distributed, oval nuclei, yet in spite of this structural abnormality the cells have absorbed blood pigment and reduced it in part to hemosiderin. Yet in spite of these good intentions no restitution of the function of the nephron as an excretory organ has occurred when the distal convolution, as shown in Plates 5, 10, 19, 23 and 25, is the seat of an irreparable disruption.

Recovery of a structural renal status compatible with adequate function therefore becomes a complex matter when the problem is considered, as it must be, from the viewpoint of "restitution of nephrons" and not "regeneration of the kidney tubules" (19). The detail of three clinical cases may be given to illustrate the varied course of the reparative processes.

The first two examples form an almost perfect experimental examination of the problem of repair in acute renal failure related to toxic injury. We are indebted to Drs. E. S. Breed and C. F. Baxter for their functional data which they will publish in detail at a later time, to Dr. R. S. Hotchkiss for

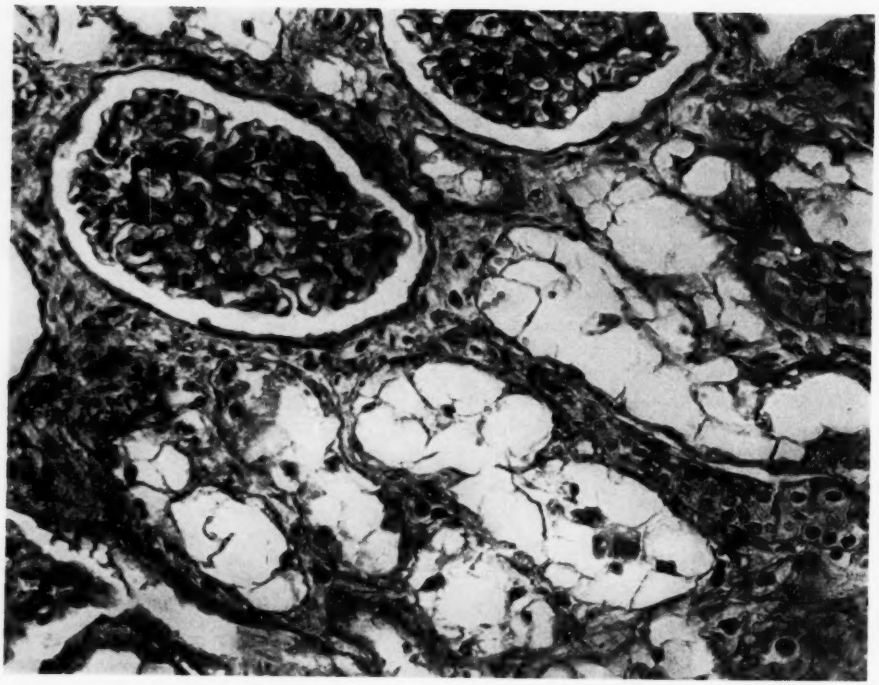


FIG. 3. DI-ETHYLENE GLYCOL POISONING—SECTION OF KIDNEY OF W. R. WHO DIED ON THE 12th DAY

Cross sections of periglomerular coils of proximal convolution showing complete vacuolar necrosis of the renal epithelium. There is no regeneration or significant interstitial inflammatory reaction. *Cf.* Plate 22 for dissected specimen; also Plate 44. Magnification 250 ×.

the clinical details and to Dr. Milton Helpern for the anatomical material.

Two young, apparently healthy prisoners, W.R. and D., in a penal institution obtained surreptitiously a supply of what was described as an anti-freeze mixture. How much was drunk and for how long continued is not known but after eight days W. R. was admitted to the hospital with bilateral kidney pain and shortness of breath; red cells and albumin were found in his urine. His blood pressure was 170/100. On the 12th day his PAH, O_2 and inulin extraction ratios were all 0; the BUN was 266 mg. per cent. During four hours 0.02 cc./min. of urine was collected. The blood K was 8.85 m.eq./l. Decapsulation was attempted during which operation he died of cardiac arrest.

D. came to the hospital on the day following his companion's entrance with essentially similar clinical findings. On the 11th day his urine flow was 0.035 cc./min. An "artificial kidney" reduced his blood pressure from 200/80 to 110/70, his NPN from 300 to 180 mg. per cent., and his blood K from 8 to 5.19 m.eq/l. The next day the extraction ratios of PAH, inulin and O_2 were 0. That afternoon a decapsulation was done and a biopsy obtained from the right kidney. The patient did fairly well postoperatively; on the 16th day he was put on the artificial kidney again and his blood K reduced from 8.6 to 5.5 m.eq/l. At this time NPN was 324 mg. per cent. The urine output increased gradually from 89 cc. in 24 hours on the 17th day to 1151 cc. on the 21st day. His inulin clearance was then 1.92 cc./min., creatinine 1.16/min. and PAH 1.31/min. Three days later he became disoriented and died.

The kidneys were therefore available from two individuals who took the same nephrotoxic agent, di-ethylene glycol as subsequent histological examination proved, at the same time. One (W. R.) died on the 12th day at what might be described as the height of renal damage and in renal failure. A biopsy was taken from the other (D.) at approximately the same period after the poison-

ing, thus allowing a comparison of the anatomical lesion in the kidneys of the two cases at the point of its maximum development. The individual, D., showed a functional recovery so far as water excretion was concerned, but died of renal failure 24 days after taking the poison.

Histological sections of the kidney of W. R. who died on the 12th day showed the typical vacuolar epithelial alteration caused by di-ethylene glycol in sections of what are apparently proximal convolutions; there were casts and debris and other alterations in the other portions of the nephron. There was no evidence of epithelial regeneration (Figure 3).

Dissections of nephrons showed the full development of the renal lesion with no evidence of repair. In Plate 22 is shown the glomerulus and the first portion of a proximal convolution; the epithelium of the tubule is completely disarranged; the vacuolar change is not evident in the thickness of the dissected specimen which was purposely heavily stained in order that the protoplasmic remnants of the epithelium would be visible. The dissected specimen shows quite clearly that the damage is more than a nephrotoxic epithelial lesion, for the basement membrane has been destroyed along the greater part of the preparation and at several places in the course of the tubule typical disruptive tubulorhexis is evident. In ascending limbs and distal convolutions (Plate 23) the disruptive tubular lesions are also present along with casts and diffuse basement membrane damage. The epithelium of the collecting tubule, however, is intact though its lumen contains irregular collec-

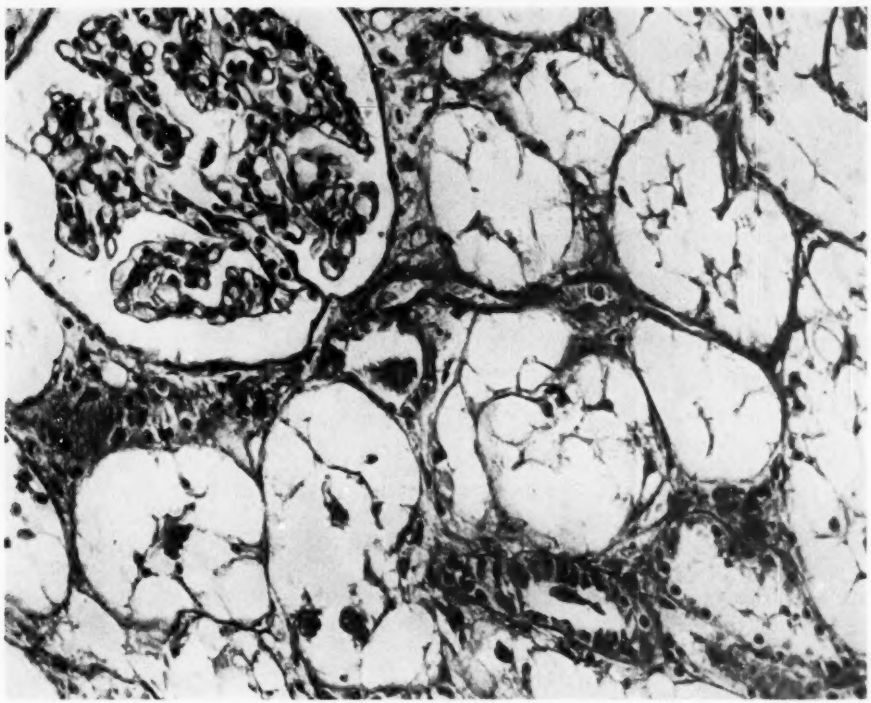


FIG. 4. DI-ETHYLENE GLYCOL POISONING—BIOPSY FROM THE CORTEX OF THE KIDNEY OF D. ON THE 11th DAY

Note that the lesion is identical with that of the other case shown in Figure 3. Magnification 250×.

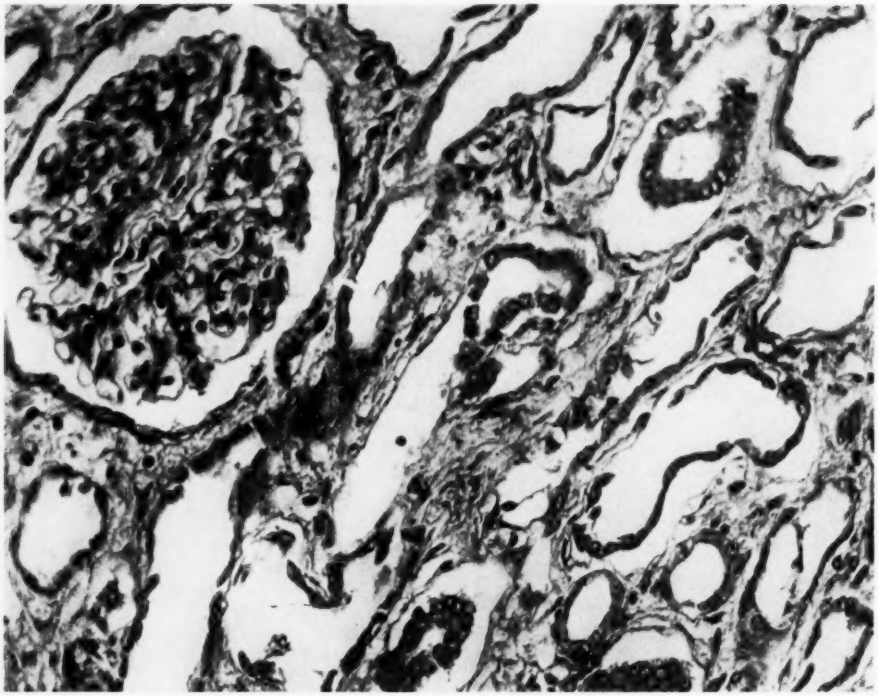


FIG. 5. DI-ETHYLENE GLYCOL POISONING—THE KIDNEY OF D. AT AUTOPSY 13 DAYS AFTER THE TAKING OF THE BIOPSY OF THE PRECEDING FIGURE

No vacuolated necrotic cells remain, the original tubular lining being completely replaced by the proliferation of a flat epithelium. There was, however, no significant return of tubular function and the lack of restitution of a nephron structure compatible with function is seen in Plates 24 and 25. Magnification 250×.

tions of cellular debris and casts. The over-all picture of the renal lesion may therefore by summarized as a combination of extensive nephrotoxic and disruptive tubular damage with no regeneration or repair.

Histological sections of the biopsy taken on the 11th day from the kidney of D. showed widespread and acute lesions identical to those observed in W. R.; this observation is of importance as it shows the degree of damage in the kidney of this individual 13 days before his death (Figure 4). Histological sections taken from the kidney post mortem, that is, approximately three weeks after the ingestion of the poison, showed none of the acutely damaged vacuolated cells seen in the biopsy in the cross sections of proximal convolutions. These tubules were considerably dilated and completely lined with a flat, atypical, regenerating epithelium which in appearance had reformed the tubule wall. In cross sections of other portions of the nephron, tubular damage and casts were frequent. In the sub-cortical region and extending deep into the medulla there was a heavy infiltration with inflammatory round cells and leucocytes (Figures 5 and 6).

Dissections of proximal convolutions present an entirely different aspect of the epithelial regeneration from what was apparent in histological section, for it can be seen at a glance that the tubule wall is neither reformed nor a continuous

lumen reestablished, which is the only sort of lumen that has functional significance. The tubule is greatly dilated and its contours are remarkably irregular. This irregularity is due both to sudden constrictions (A) scattered along its length and to sudden fingerlike pouches and protrusions (B) which are formed of dark staining bands and strips of regenerating renal epithelium. Moreover regenerating cells are not confined to the wall of the tubule, but grow in random whorls that double back into the lumen (C) and at times form valve-like flaps and irregular masses which impinge upon and obstruct it (Plate 24).

In the ascending limbs and distal convolutions there is also extensive disruptive tubular damage, extensive cast formation and irregular repair. The sheets and bands of dark staining regenerating epithelium are clearly seen; it will be noticed that they are exceedingly patchy in distribution and that the tubule wall, even where regeneration is most active, is far from reestablished. The dark black segments are portions of relatively intact tubule filled with casts. Frank disruptions of the tubule wall with surrounding ingrowths of inflammatory granulation tissue are present (Plate 25).

To summarize, it is clear that in this kidney which showed grave structural damage on the 11th day, and of which, from the appearances noted in histological section, it would have been said that extensive "tubular regeneration" had occurred by the 24th day, there was little evidence of an adequate reconstitution of functional nephrons in spite of the exuberance of epithelial regeneration.

Referring back to the examples of toxic renal injury due to sublimate and potassium chlorate,

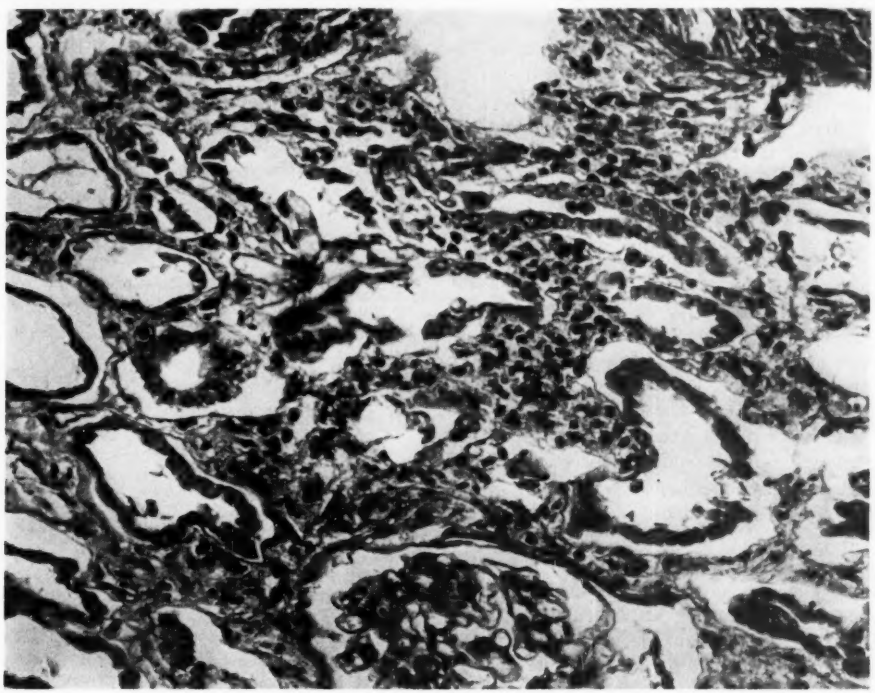


FIG. 6. SAME SECTION AS FIGURE 5 SHOWING CROSS SECTIONS OF DISRUPTED TUBULES WITH HEAVY INFLAMMATORY REACTION IN THE INTERSTITIAL TISSUE. Magnification 250 ×.

similar difficulties in the repair of the disruptive tubular lesions may be noted. In Plate 16 the large disruption in the proximal convolution is filled with masses of dark staining, atypical epithelial cells that protrude into the irregularly dilated lumen and, as a result of the disintegration of the basement membrane, form isolated islands of proliferating epithelium rather than a reconstituted tubule wall. In Plate 19 the swollen disruption in the distal convolution is only partially lined by sheets of epithelium, and these, lacking the support of a basement membrane, stream out irregularly into the tubule lumen.

As evidence of the permanency of the damage that is inflicted on the individual nephrons by the disruptive tubular lesion, an example of repeated episodes of renal damage over a period of years may be cited. Our material was obtained from a case of paroxysmal hemoglobinuria reported by Drs. Ralph Sussman and Herbert Kayden (20). After 11 typical attacks over a period of two years, an extraordinarily severe seizure resulted in oliguria and nitrogen retention with death on the 12th day. The typical lesions of tubular disruption and heme pigment casts were described by Dr. Alfred Plaut and the authors point out the similarity of the renal condition to "post-transfusion and crush injury nephrosis." Of interest to our immediate problem, the histological examination by Dr. Plaut showed not only typical acute tubular lesions but also "a diffuse fibrotic process with numerous scars . . . apparently remnants of renal injury resulting from 11 previous hemolytic episodes. It should be noted that no vascular changes were reported to suggest a vascular cause for the renal lesions."

In the more dense fibrotic scars which were scattered through the renal cortex only remnants of nephrons could be found by dissection. These consisted of atrophied bits of what could still be recognized as proximal convolutions attached to shrunken, fibrous glomeruli. In the looser fibrous tissue at the periphery of the scars better preserved but markedly deformed nephrons were found. Plate 26 shows an irregularly atrophic proximal convolution in which are seen several tubulorhextic disruptions surrounded by adhesions of granulation tissue that resulted from the terminal acute attack. In Plate 27 is seen a badly damaged ascending limb and distal convolution which, probably as a result of peripheral obstruction, shows a huge irregular cystic dilation. Numerous diverticula, the origin of which bears no relation to the renal lesions, are present.[3]

To summarize our findings regarding the reparative process: restitution of function in kidneys of Acute Renal Failure associated with traumatic and toxic injury would seem to depend chiefly on the recovery of functional normality in nephrons which have never suffered the fully developed disruptive lesion. In the case of poisoning, where nephrotoxic tubular lesions with an intact basement membrane predominate, epithelial regeneration can reconstitute the tubule of the nephron with relative ease; if there is extensive tubulorhextic damage, as there is in fatal poisoning, the reparative problem is less simple. Then, as in the case of "the crush" or "shock" kidney where disruptive tubular lesions prevail, recovery of renal function depends on the restitution of functional normality in nephrons which have never suffered the full brunt of the disruptive injury. The peculiarly spotty distribution of these lesions among the nephrons, some of which show no significant damage in the most grievously altered kidney, makes this eventuality possible.

Summary of the structural alterations in human kidneys in Acute Renal Failure

Before proceeding to an examination by the experimental method of aetiological problems, a brief summary of the structural alterations seen in the human kidney in Acute Renal Failure related to traumatic and toxic injury should be useful to fix clearly in mind what sort of lesion is to be reproduced.

The distinctive disruption of tubules described by Dunn, Gillespie and Niven is characteristic of all members of this group. This lesion, for which the name tubulorhexis has been suggested, occurs in all parts of nephron indiscriminately from the

[3] These objects, found not infrequently in normal kidneys and only on the distal convolution, may become so large as to have the configuration of glomeruli (*cf.* insert, Plate 27); they have on occasion been described as such in dissected specimens and so led to much perplexity as to how glomeruli could become attached to collecting tubules. In histological sections they may appear as gland-like structures independent of the tubules and, with their contained colloid-like material, have been interpreted as renal "endocrine" elements.

proximal convolution to the collecting tubule. Its distribution is entirely random both in the nephron and among the nephrons; even in the kidney that has failed some nephrons are found essentially undamaged. The lesions show no constant association with either casts or accumulations of heme pigment; the lumen of the distal nephron commonly contains these materials with no damage to the tubule wall.

In kidneys from cases where poisoning has been the clinical cause of renal failure there is also found besides the disruptive lesions tubular damage of a nephrotoxic nature; this is located in proximal convolutions and is evenly distributed throughout all the nephrons. Such lesions are absent or minimal in cases where the renal damage follows shock resulting from traumatic injury.

THE REPRODUCTION OF THE LESIONS OF THE KIDNEY OF SHOCK BY EXPERIMENTAL PROCEDURES

Now that the lesions observed in the human kidney of shock have been described we may return to our earlier stated problems of aetiology, pathogenesis and clinical correlation. It is clear at once, however, that these questions present themselves in a very different form when they arise from the consideration of the description of the structural status of the damaged kidney that we have given rather than on the basis of the current designation of the lesion as a "lower nephron nephrosis." The problems to be put to experimental test are therefore quite different from those which have been examined by previous investigators, and this is particularly true when the experiment is directed toward the reproduction of the human type of lesion in the kidney of animals. *A priori*, one could be reasonably certain that no such disturbance as an induced ischemia could produce lesions limited to the "lower nephron" for the anatomical characteristics of the renal circulation and the topographical arrangements of the nephron make such an eventuality impossible. As has been suggested in our introduction, the frustrations and difficulties of experimenters in the past were, therefore, due to the fact that questions were asked that had no answer. With the knowledge that proximal convolutions must be damaged if the human lesion is to be reproduced and that in fact any or all parts of the nephron may be involved, we can approach the experimental verification of the thesis of ischemic damage with a new and more hopeful attitude. This is particularly true since there would seem to be little chance that experiments based on a hypothesis of toxic damage would be successful, for it is apparent from our descriptions that the essential disruptive tubular lesion of the human kidney is different, in both its nature and location in the nephron and in its distribution among the nephrons, from what is produced experimentally by nephrotoxic poisons. No toxic substance reaching the kidney by the blood stream could conceivably cause such random damage as we have described in such functionally disparate parts of so many independent organs.

Among all the factors that have been suggested as a cause of the renal lesion, ischemia stands out as the disturbance *par excellence* which produces random and scattered damage. This is especially so when the circulation fails in small, widely distributed, terminal arteries and arterioles; an example of its effect is seen in the kidney itself where arteriolar-sclerotic fibrous scars, irregularly scattered through the renal cortex, encroach upon and surround atrophied portions of the nephrons at random. So close is this analogy drawn from his general experience, that the pathological anatomist might have little hesitation in deciding our problem on the basis of induction from his structural observations of the human renal lesions alone if the experimental verification of this conclusion were not possible.

As a matter of fact the recent development of a visual technique has made it possible for the morphologist to observe directly the circulatory status of the kidney and, as is desired in our problem, to actually see the pattern of an ischemia as it existed in the experimental animal at the moment prior to its sacrifice. Schlegel and Moses (21) have shown that the fluorescent dye, thioflavin S, when injected intravenously in small amount, diffuses into the wall of the arteries and arterioles and glomeruli and is there retained for a sufficient time so that if the kidney is promptly removed and viewed at low magnification under ultra-violet light all the glomeruli and vessels which had been receiving blood containing the dye stand out in brilliant secondary fluorescence against the darker background of the renal tissue. We

shall use this technique at a later point in our investigation and will at that time describe more fully the technical details. For the present, one of Drs. Schlegel's and Moses' original illustrations of a normal kidney of a rabbit showing the arteries, arterioles and glomeruli that were receiving blood at the moment of its death is seen in Figure 7A. The even distribution of the circulatory pattern is most beautifully demonstrated and in contrast, Figure 7B, taken from the same report, shows the pattern of the renal ischemia that followed the intravenous injection of 1/10,000 adrenalin. Irregular areas of renal cortex are seen in which the patent and fluorescent vessels and glomeruli stand out in brilliant contrast to the patches of dark ischemic tissue where the circulatory bed had received no dye. If one visualizes the distribution of the constituent nephrons on this spotty background of ischemic cortex one can anticipate how the random pattern of tubular damage that we have described in the nephrons of the human kidneys of shock might develop. (Figure 8.)

* * * * * *

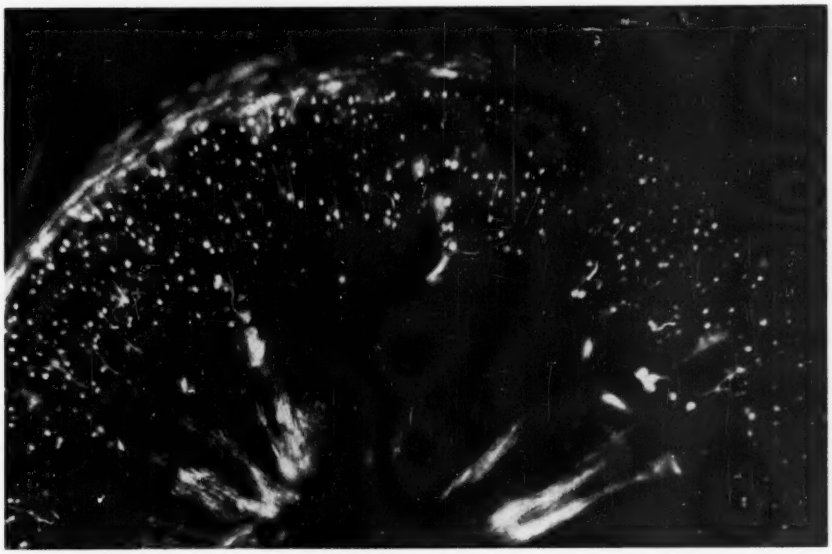
Our examination of the structural lesions in the kidney of experimental shock was made on material from the extensive studies of Drs. D. D. Van Slyke, R. A. Phillips, V. P. Dole, P. B. Hamilton, K. Emerson, Jr. and R. M. Archibald, on the functional disturbances that occur in the kidney in this condition (5, 22).

In these experiments on dogs the functional effects of the renal ischemia which is known to be part of the general phenomena of shock in man were examined under varying conditions. To determine the effect of duration of the ischemia, clamps were placed on the renal artery so that blood flow to the kidney was arrested for periods up to three hours and longer. In animals whose renal artery was clamped three hours, the kidneys after a few days of renal inadequacy ultimately recovered their functional capacity; those whose artery was clamped longer died in renal failure. Analogous functional disturbances were observed in dogs in shock when the renal blood flow, estimated by hippurate clearance, had been reduced as a result of a hemorrhage or trauma below 1 per cent of its initial normal rate for a period of four hours. These investigators found that in general the progressive effects of ischemia on the kidney, both in the experimental animals and in human cases, could be divided into three stages: (*1*) reduced renal function without functional evidence of damage to nephrons; (*2*) more severe reduction in renal function with functional evidence of reversible damage to nephrons; and (*3*) the development of a progressive uremia and death with apparently irreversible damage to nephrons. The first two stages could be produced in dogs by shock resulting from trauma or hemorrhage; the production in the dog of the third stage usually required the clamping of the renal artery for at least four hours.

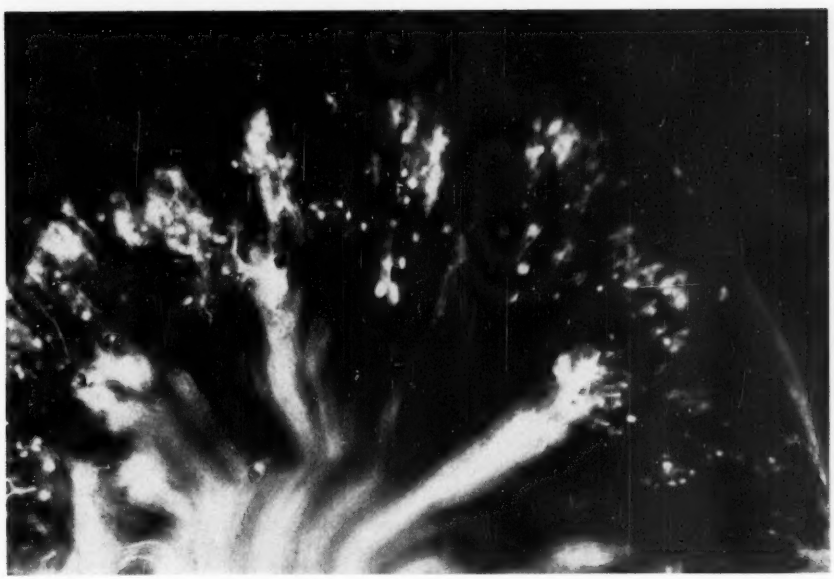
The kidneys of representative animals from these investigations were studied by us in histological section and by microdissection. It can easily be understood from a consideration of the precarious situation that must have obtained in experiments of the sort described (where the animal is in severe shock or is subjected to such relatively grave experimental procedures as a prolonged clamping of his renal artery and yet must be maintained so as to respond to elaborate functional examinations), that it cannot be hoped that every experiment will approximate the delicate balance that determines the status of the human kidney in naturally occurring shock. Many of the kidneys, especially those whose renal artery had been clamped four hours, showed gross structural damage far beyond that ever seen in the kidney of shock in man and in other experiments, conversely, not enough structural damage was produced to be comparable with the fatal human renal lesion. The grossly damaged kidneys, as we shall point out later, were useful, however, as their exaggeration confirmed conclusions derived from the fully successful experiment where both functional and structural lesions reproduced the syndrome seen in the human kidney of shock.

The effect on the kidney of direct ischemia produced by clamping the renal artery

We have found that nephrons from kidneys, the renal artery of which has been clamped four hours or longer, cannot be dissected because the basement membrane of the necrotic tubules, though still visible in sections, is also "necrotic," with the result that the tubules disintegrate completely on maceration and only short remnants and debris can be isolated. It is noteworthy, therefore, that the



A



B

FIG. 7. FROM SCHLEGEL AND MOSES—VISUALIZATION OF KIDNEY BLOOD VESSELS (21)

A, the pattern of the normal circulation in the rabbit kidney; B, the patchy ischemia resulting from the action of adrenalin. Reproduced by permission of the authors and publishers.

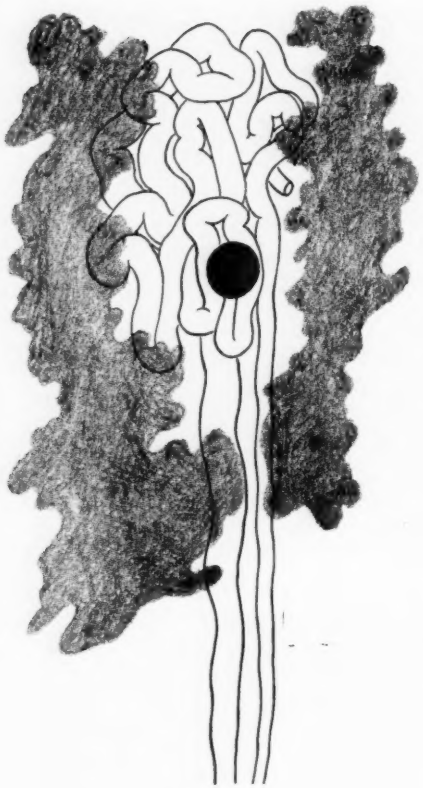


FIG. 8

tubular lesion in these exaggerated instances of experimental ischemic damage show the essential characteristic that was noted in the tubulorhexis of the human case, *i.e.*, dissolution of the basement membrane as well as epithelial necrosis.

Considerably less ischemic damage is shown in the following experiment (dog K29). Under nembutal anesthesia the area of the renal vessels was exposed on the left side and two rubber covered serrefines placed on the left renal artery where they remained for 66 minutes. The right kidney remained undisturbed *in situ*. Seventy-two minutes after the removal of the clamps a 350 cc. infusion was given consisting of 50 cc. of the animal's red cells laked with 200 cc. of distilled water with added salt to isotonicity and 50 cc. of the animal's plasma. As one kidney had remained intact in this experiment no functional examinations were made. The animal was killed five days later.

Histological sections of the right kidney showed no lesions; in the left kidney there were irregularly scattered areas of tubular destruction in which proximal convolutions were prominent; approximately one-half of the cortical tissue was thus involved.

Dissections of nephrons showed a peculiarly localized nature of tubular destruction: rarely was the greater part of any portion of a nephron entirely involved. In Plate 28 is shown a complete proximal convolution. Its first portion is completely destroyed, the basement membrane has disintegrated and its epithelium is reduced to debris. The mid-portion then appears remarkably well preserved; even the mitochondrial rodlets, the most sensitive indicator of cellular damage, can be seen and the usual cellular pattern of the unstained nuclei of the renal epithelium is clearly apparent. At the beginning of the medullary segment scattered areas of epithelial necrosis appear which become progressively more extensive until complete epithelial disintegration is observed in its terminal part which narrows to the thin limb of Henle's loop. It should be noted that in the severely damaged terminal portion of the convolution the basement membrane, so clearly seen in the normal middle portion, had disappeared.

A typical lesion in the distal portion of the nephron is shown in Plate 29. The contours of the ascending limb to the left are fairly well preserved but throughout its length irregular collections of cellular debris are seen in the tubule lumen. The distal convolution is filled and irregularly distended with deep black, consolidated haemoglobin casts that were positive to the benzidine test (A) and at (B) a typical disruptive lesion of tubulorhexis is observed. Crystal-like deposits of heme pigment are well shown at this point (B^1). It can be observed that from here on the distal and connecting tubule are markedly distended with a solid coagulum of heme pigment yet the tubule wall is intact.

This experiment is of interest in showing that though in histological section the severity of the damage in the proximal convolution masks its essential identity with what is observed in the kid-

ney of human shock, an examination of the lesion in the isolated nephrons shows not only the same type of limited, disruptive tubular damage but also the same random involvement of scattered stretches of nephron from proximal to distal convolution that is so characteristic of the human lesion.

In experiment K26 an even closer approximation to the human lesion was obtained. Under nembutal anesthesia the right kidney was removed and a serrefine placed on the left renal artery for 60 minutes. The animal remained in good clinical condition; no functional studies were made and it was killed on the fourth day after the operation.

Sections of the left kidney show none of what is commonly called "frank" necrosis of renal tubules. The epithelial cells in occasional cross sections of a proximal convolution appear desquamated or disarranged but so rare is this appearance among surrounding well-preserved cross sections that a conservative examiner would probably ignore it as some sort of fortuitous artifact. The distal convolutions and collecting tubules contain casts that stain deeply with eosine and which are benzidine positive but negative for free Fe.

Plate 30 shows a complete proximal convolution. The cellular pattern is particularly well shown; at (A) even the mitochondrial rodlets of the epithelium can be made out. At the arrows various degrees of cellular damage, from an irregularity of the mitochondrial pattern to complete necrosis with disruption of the tubule wall, are evident in short isolated segments of the convolution. At (B) are seen short segments of tubule where the basement membrane is destroyed and the necrotic epithelial cells are resolved into dark stained debris. In Plate 31 three distal convolutions join a collecting tubule on the left. In the distal convolutions and collecting tubule (A) can be seen the infrequently mentioned dark staining intercalated cells peculiar to this portion of the nephron. There are many small diverticula on the upper portion of the collecting tubule. There are no casts or damage except in the lowermost distal convolution. At (B) the tubule is disrupted and filled by a black stained plug of heme pigment; at (C) the lumen is packed with similar material but the epithelium is intact.

To summarize these experiments it may be said that under proper conditions of degree and duration, direct ischemia of the kidney following clamping of the renal artery reproduced the essential structural lesions that are observed in the human kidney in shock. In the instances where either the degree or the duration of the ischemia was extreme, the extensive structural damage still showed in exaggerated form the two characteristics of the renal lesions that are seen in human kidney of shock, namely irregularity of distribution of tubule damage, both within the nephron and throughout the kidney as a whole, and the complete destruction of limited stretches of tubule and basement membrane scattered along the course of a well preserved normal nephron.

The effect on the kidney of ischemia produced by renal vasoconstriction following trauma

The second group of experiments was analogous in the manner in which renal ischemia was produced to what occurs in the "crush kidney" of man, namely a renal vasoconstriction following as a reflex to trauma of the muscle masses of the limbs. Conversely to the previous experiments where exaggerated effects were more easily produced, in these the difficulty of a successful demonstration lay in the production of a sufficient degree and duration of renal ischemia to produce structural lesions and in the necessity at the same time of maintaining the animal in a physiologically adequate condition that would allow the performance of the functional tests (in particular hippurate extraction) that measured the degree of functional damage and renal blood flow. The details of these experimental procedures and the rationale of the functional determination of renal blood flow are given *in extenso* in the publication of Phillips, Dole, Hamilton, Emerson, Archibald and Van Slyke (22). The kidneys examined were from experiments similar to those shown in Table IV of that article: unfortunately the kidneys from the identical dogs of this table were not available for structural examination, but the functional findings in those whose structural status is here reported were similar. The rate of hippurate excretion during the course of each experiment will be given as an indication of the degree and duration of renal ischemia that existed in the kidneys.

1. Minimum functional and structural damage (K33). Under deep nembutal anesthesia, with the customary priming dose and sustaining infusion of PAH, trauma to the legs was begun at minute 92 of the experiment. At 94 the PAH excreted had fallen from 5320 γ/min. to 709 γ, at minute 119 to 11 γ, and at 131 to 1 γ/min. The trauma was interruptedly repeated from minutes 134 until 301, and during this period the average of PAH was 4.7 γ/min. A transfusion of the dog's own blood at minute 303 was followed by a rise of excretion to 33 γ/min. and at minute 397, to 2890 γ/min. On the fourth day, though the animal's clinical condition was good, it was sacrificed. The blood urea N preceding the experiments was 15 mg. per cent. It rose to 24.8 on the second day and was 14.5 before death. Judging from the functional examination it might be said that although there was a period of extreme renal ischemia for 200 minutes the functional damage was reversible and not severe.

Histological section of the kidney showed only slight evidence of damage, much less than that seen in even the least damaged kidneys of the previous experiments where the renal artery was clamped. Benzidine positive casts, however, were frequent in the distal convolutions.

Dissections of nephrons showed the majority to be well preserved; many, however, showed the lesions to be described.

The lesions in the damaged nephrons were entirely similar in distribution throughout the nephron and in their nature to those seen in the former experiments of clamping the renal artery and to those that we have described in the human cases. Plate 32 shows a typical complete proximal convolution. The normal epithelial pattern of clear nuclei in a dark background of cellular granular protoplasm exists throughout the greater part of the convolution. At (A) the tubule is swollen, the epithelium entirely necrotic and the basement membrane disrupted. The detail of these lesions is shown in the inserts of Plate 33. The arrows indicate where lesser degrees of similar damage may be seen. The terminal segment contains, as it normally does in the dog,[4] a considerable amount of fat which stains so black that cellular detail is obscured. In Plate 33 is shown a typical ascending limb and distal convolution. The first portion of the former contains isolated casts of black stained heme pigment; its upper portion and all of the distal convolution is distended and filled with a solid continuous heme stained coagulum. The cellular detail is thereby completely obscured, but the damage to the basement membrane of the distal convolution is evident from the adhesions which surround the tubule wall.

2. Severe functional and structural damage (K32.) The experimental procedure was essentially similar to the preceding experiment. At 21 minutes the priming dose of PAH was begun. At 49 minutes PAH excreted was 3100 γ/min. Trauma was begun at 70 minutes; at 80 minutes excretion had dropped to 1660 γ/min. The trauma was repeated at intervals and at 159 minutes PAH excretion was 3.0 γ/min. and fell progressively to 1.5 γ/min. At 305 minutes 1000 cc. of a whole blood transfusion was begun; to the blood were added 35 cc. of laked blood cells. The animal responded well. Before the experiment the blood urea nitrogen was 10.9 mg. per cent. On the fourth day it was 25.4 and on the seventh day, 79.4. The animal, having convulsions, was sacrificed.

Histological sections of both kidneys showed moderate but definite damage to tubules. An occasional cross section of a proximal convolution could be found with definite necrosis of epithelial cells. There were many heme pigment casts, positive to benzidine but negative to the Berlin Blue reaction, in distal convolutions and definite epithelial necrosis in "straight tubules" which might be either ascending limbs or medullary segments of proximal convolution.

On dissection a relatively large number of nephrons, definitely the majority, showed varying degrees of damage. In Plate 34 is seen a com-

[4] The presence of fat in the terminal portion of the proximal convolution of the normal dog nephron is well-known to anatomists (23) but less so to some experimentalists. As a result there quite regularly appears in the literature correlation of various procedures with the experimenter's discovery of this normal anatomical fact. In the last two years the senior author can recall three such instances; two in which dietary factors and one in which a neurological lesion were implicated. There will doubtless be more, so that the general reader may care to keep this bit of elementary histology in mind for future critical use.

plete proximal convolution. The first two thirds are fairly well preserved though stretches are seen where the cell pattern is disturbed. Beginning in the terminal medullary segment (A) there is frank necrosis of epithelial cells and disintegration of the basement membrane with resulting peritubular adhesions. From this point on to the end of the convolution is seen an extensive and complete necrosis of the tubule. The detail of these lesions is shown in Plate 35 at a higher magnification.

Ascending limbs and distal convolutions also frequently showed typical tubulorhextic lesions (Plate 36). The cellular pattern of the lower portion of the ascending limb is visible; its upper third contains black stained heme pigment casts and it passes into a distal convolution that also is distended and plugged with a similar material. The wall of the distal convolution is disrupted and there are the usual peritubular adhesions.

To summarize: in these experiments where renal ischemia followed upon shock produced by trauma to muscle, lesions of the nephrons were produced similar in their nature and distribution to those that were found in the human "crush kidney."

One point stands out more clearly in the experiments than in the human examples of the renal lesion, for in them it is plainly evident that the degree of damage in the kidney of shock is largely a function of the number of nephrons affected by the ischemic insult and not of the severity of the lesion in the nephrons *per se*. The few nephrons of the first experiment where renal function was not greatly reduced were damaged as severely and in the same manner as the many damaged nephrons of the third experiment in which renal failure resulted. This is in striking contrast with the situation in the kidney of widespread nephrotoxic tubular damage where, since all nephrons are universally affected by the toxic insult, it is the degree of nephron damage that determines the total functional result.

Another apparently minor point, but one to which we shall later refer in our discussion, is established by the experimental procedure. This is the remarkable cytological normality of the epithelial cells of the tubule in regions other than where ischemic disruptive tubulorhexis has occurred. The immediate fixation of the tissues that is possible in the experiment allows the observation of such detail as the intactness of the mitochondrial rodlets in the cells throughout the nephron immediately up to the point of disruption (Insert, Plate 28). There is therefore no evidence of any toxic damage in the experimental lesion produced by the identical mechanisms that are the cause of the "crush" kidney in man.

The heme pigment metabolism in the kidney of shock

We have previously described the occurrence of heme pigments only as they appear in casts in the distal segments of the nephron. These deposits lie in the lumen of the tubules and therefore outside the kidney tissue and from their histochemical reactions, *i.e.*, positive benzidine test and negative Berlin Blue reaction, have undergone no apparent alteration in their chemical structure since they give the same reactions to these tests as does haemoglobin. As it is well known that heme pigments filtering through the glomerular membrane under a variety of circumstances are absorbed by the epithelial cells and are there broken down to simpler degradation products, it would seem warranted to consider these processes as a special case of the metabolic mechanism by which the kidney handles proteins (9). Recent studies of these activities in the case of blood pigments may be found in the work of Rather (18) and Lippman, Ureen and Oliver (24).

In our earlier discussion concerning the finding of heme pigments in the casts of the human "crush" kidney the comment was made that it seems singular that so little mention is made in the current literature of these processes in the proximal convolution. The presence of the pigment in the distal portion of the nephron is proof that the cells of the proximal portions must have been in contact with these materials, so that the absence of the usual absorptive activity in the proximal convolution requires an explanation. Considerable light is thrown on this anomalous situation by an examination of the material from the experiments of Van Slyke and his co-workers and comparison of these findings with those of the human material.

The full picture of the renal metabolism of the heme pigments is seen in those experiments where the more natural and moderate mechanisms of

reflex vasoconstriction following trauma were the cause of ischemia and nephron damage. In these experiments the ischemia, though extreme and of more than one hour duration, was not absolute as it was in those in which the renal artery was clamped. Three potential sources from which heme pigments may enter the tubule lumen are evident in the experiments; namely, as a result of the glomerular filtration of haemoglobin free in the circulating blood, from the transfusion of whole blood even when not followed by any perceptible clinical reaction and from the entrance of myoglobin and possibly also haemoglobin as a result of muscle trauma. In experiment K32 the urine was noted as "blood tinged" 28 minutes after trauma was begun and 207 minutes before a transfusion was given. A fourth source may also be mentioned in passing though there is no direct evidence of it in the experiments, namely the passage of red cells through glomeruli made more permeable by ischemic damage followed by haemolysis of them in the tubule lumen. At least there seems no other source of the few benzidine casts found in experiment K26 where the renal artery was clamped but no transfusion or trauma to muscles occurred.

In histological sections of all of the trauma experiments (K31–33 and K35) whether laked blood or an apparently reactionless whole blood transfusion had been given, the epithelial cells of those parts of the proximal convolutions which had escaped damage, a considerable portion as judged by the intact mitochondrial pattern seen in Plate 32, were filled with fine benzidine positive droplets. There was also, as is always observed in normal renal cells absorbing heme pigments, a light diffuse tinging of the protoplasm by the benzidine reaction. In the occasional cross section that showed damage in the form of disruption or desquamation of epithelial cells only a very light diffuse staining was evident and few if any droplets. In the lumen of the proximal convolutions there was also seen granular detritus or debris that was benzidine positive, but in straight tubules in the outer stripe of the medulla, presumably ascending limbs, and in the periglomerular loops of the distal convolution, deep brownish yellow stained, well-consolidated casts were present. There were also many benzidine positively reacting casts in collecting tubules.

As all these animals had lived several days, the degradation of the heme pigments could be followed. The Berlin Blue reaction was strongly positive in all the cells of the proximal convolution that the benzidine stain had showed to contain heme pigment. The free Fe existed either as discrete granules or as a bluish tinge of droplet-like structures. Within the lumen of the proximal convolution the debris was faintly positive for free Fe, but the massive well-consolidated casts in the ascending limbs, distal convolutions and collecting tubules were negative.

In those experiments where an absolute ischemia for three or more hours had followed a clamping of the renal artery no evidence of heme pigment absorption or degradation was found in the severely damaged cells of the proximal convolution even when copious infusions of laked cells or whole blood had followed the unclamping of the artery. The necrotic cells of proximal convolutions were occasionally tinged a faintly diffuse but perceptible yellow, indicating that heme pigment in solution had seeped through the dead tubule wall, and in such kidneys there were many heme pigment casts in ascending limbs, distal convolutions and collecting tubules that were strongly positive to benzidine. It seems therefore reasonable to conclude that the extreme structural tubular damage, including necrosis, of great stretches of proximal convolution had abolished the functional activity of pigment absorption. As a consequence of this absence of absorption there resulted a negative reaction of the proximal convolutions to the Berlin Blue test which is the indication of heme pigment degradation.

In certain experiments where transfusions or haemoglobin infusion followed one hour after total ischemia from a clamping of the renal artery, lengths of proximal convolution remained undamaged at least in the structural sense and yet in these structurally intact cells there were observed only traces of a diffuse positive reaction with benzidine and no well formed droplets. In the lumen of the lower reaches of the nephron the consolidated heme casts were strongly positive. As might be expected, no free Fe resulting from intracellular pigment degradation was found in the cells or lumen of any region of the nephron. The cellular damage responsible for this failure of cellular activity was invisible to the methods of

examination that were available and in this restricted sense the lesion may be said to be "functional" (25).

To summarize: in all the experiments, whether the ischemia was the result of clamping the renal artery or of reflex renal vascular spasm following muscle trauma, heme pigment flowed through proximal convolutions and was consolidated into casts in the distal nephron. Such pigment at no time was intracellular and therefore was not broken down to show free Fe. In the experiments where the ischemia was the result of the more natural and moderate mechanism of reflex vascular spasm and where large stretches of proximal convolution remained undamaged, absorption of heme pigment in droplet form and subsequent degradation to a Fe free form was observed. In the more drastic and total ischemia following clamping of the renal artery where proximal convolutions were extensively damaged no absorption of heme pigment and consequently no degradation products occurred. Occasionally proximal convolutions, which by present methods of examination appeared structurally intact, contained no pigment.

The experiments therefore supply a morphological demonstration of variations in the damage that may occur to one of the functions of the nephron, namely protein absorption. The phenomena of heme pigment metabolism differ strikingly in their manifestations even in the relatively uniform group of experimental animals. It is therefore not surprising to note even more remarkable variation among the examples of the human shock kidneys, where conditions of origin and subsequent clinical course were extremely varied. The one manifestation previously noted by all writers was consistently found in our material; that is, the presence of heme pigment positive to benzidine and negative to Berlin Blue test in the casts of the distal convolution and collecting tubules; it was the rare example where either the benzidine or Berlin Blue reaction showed that any of this material had been absorbed in the preceding proximal convolutions. In only two examples was the typical and complete picture of heme pigment metabolism present. One of these was a case of transfusion reaction following shock after prostatectomy and the other a case of blackwater fever. In both instances not only were the benzidine positive casts present in the distal nephron but droplets positive to both benzidine and the Berlin Blue reaction were seen in the proximal convolutions in histological section and dissected nephrons (Plate 12).

One case of blackwater fever deserves further comment. In histological section the cells of its proximal convolutions were distended with large droplets that stained heavily with iron haemotoxylin and benzidine. That this was an exaggerated example of the condition was clear from the appearance of the kidneys: the formalin-fixed tissues of the gross specimen were almost black and in sections not only was intracellular and intratubular pigment present, but even the interstitial tissue as well was impregnated with "formalin precipitate" and benzidine positive haemoglobin. Though postmortem diffusion may be in part the source of this impregnation, excessive quantities of the pigment from violently acute intravascular haemolysis might well be expected to appear in the interstitial fluid of a kidney, the nephrons of which are damaged to the point of physical rupture. We believe therefore that this case shows in visible pigmented form, that leakage of protein-containing fluid from the lumens of disrupted tubules the significance of which in the production of alterations in renal function will receive further attention in our discussion.

Our experience derived from the study of human lesions viewed in the light of the experimental findings therefore suggests that though the typical phenomena of heme pigment metabolism may occur in the shock kidney of man its manifestations are usually modified and obscured. If enough pigment reaches the tubular lumen of the proximal convolutions over a sufficient period of time and if the cells of the proximal convolutions have not been too greatly damaged, as they were for example in the experimental kidneys where the renal artery was clamped, then in addition to the distal pigment casts there may be found the evidence of tubular absorption of heme pigment and protein degradation in the proximal convolution. Renal ischemia, which we can now believe to be the essential cause of the disruptive tubular lesion in man, can therefore, as in the experimental animals, quite adequately account for the functional failure of absorption in the proximal convolutions as well as for the associated structural lesions.

AN EXPERIMENTAL EXAMINATION OF THE RELATION OF TOXIC AND ISCHEMIC DAMAGE IN THE KIDNEY OF ACUTE RENAL FAILURE

The structural characteristic of the kidney in fatal Acute Renal Failure that differentiates this condition from other nephropathies is found not in the location of tubular lesions but in their nature. We have shown that the Dunn-Gillespie-Niven lesion, to which for convenience we have given the name of tubulorhexis, occurs at random in all parts of the nephron, that it consists of a peculiar form of disruption of the entire tubule wall and that such randomness and disruptive damage can be explained as a result of ischemia. These conclusions, drawn from a study of the spontaneous lesions seen in man, are confirmed by the experimental reproduction by Van Slyke and his co-workers of the lesion in dogs by renal ischemia resulting from various procedures, including traumatic shock, an admittedly important source of the renal disturbance in man.

It has also been shown that another type of tubular damage is frequently found in the human kidney of Acute Renal Failure, namely various "degenerations" and necrosis of the epithelium of the proximal convolutions; these lesions have all the structural appearances of the action of a toxic agent.

This confusing combination in a single kidney of tubular lesions of such different nature and origin constitutes one of the major difficulties in current concepts since it lies at the very origin of the renal disturbance. The confusion may be readily cleared if one accepts what seems to the pathologist the obvious solution; namely that in certain situations there are two sorts of renal damage in the final picture, each due to its own mechanism. Renal ischemia, a result of circulatory imbalance or "shock," might well complicate a severe intoxication which has produced its usual nephrotoxic effect on the renal tubule and so produce in addition the random tubulorhextic lesions that characterize the full-blown kidney damage that is seen in certain examples of fatal Acute Renal Failure. The drinking of an anti-freeze compound, or any other renal poison, would therefore bear the same relation to the final renal status as does a traumatic crush accident, the convulsions of eclampsia or a transfusion mishap. By this concept the difference between Acute Renal Failure following poisoning and that resulting from the effects of traumatic injury and shock would lie in the fact that in the case of poisoning the effects of two renal insults are present rather than one.

This conclusion arising from the structural aspect of the lesion is in fact supported by the results of the clinical-functional study of the disturbances observed in certain chemical intoxications that conclude with the suggestion by the investigator that there may be some combination of the effects of specific nephropathic action and ischemia. Sirota (*26*), for example, in his study of carbon tetrachloride intoxication and Marshall and Hoffman (*27*) so interpret the results of their studies by clearance methods.

If it is difficult or at present impossible to distinguish such pathogenetic minutiae in the complexity of the clinical case, it should be possible to demonstrate these distinctions under experimental conditions. Certain nephrotoxic substances produce, under proper conditions, exquisitely localized tubular damage. It was shown in 1912 by Susuki (*14*) that uranium caused tubular necrosis in the middle third of the proximal convolution and not elsewhere, and this finding, a difficult if not precarious conclusion on the evidence available in histological sections, was confirmed by microdissection of complete nephrons by the senior author in 1915 (*28*).

Any morphologist who has been practically concerned with the study in histological sections of considerable numbers of animals poisoned with uranium or any other heavy metal has doubtless had the experience of seeing tubular lesions which, though obviously predominant in the proximal convolution, seem at times to involve other portions of the nephron; the ascending limb of Henle's loop, for example. Appreciating the difficulty or impossibility of exact orientation in sections, such apparent discrepancies are usually passed without comment, but do leave a certain scepticism in the mind of the observer as to the exactness of Susuki's description. It will now be shown that under some circumstances there is real cause for doubt; other parts of the nephron besides the proximal convolution can be seen to be damaged in dissected specimens. Moreover it will be shown by visual demonstration that in these kidneys there existed at some time prior to the sacrifice of the

animal a patchy ischemia and that in dissected nephrons two sorts of lesions are found; first, the distinctive nephrotoxic damage in the proximal convolutions of all nephrons and secondly, random tubulorhextic disruption in various and indiscriminate parts of some.

In the following experiments a functional disturbance in the renal vascular bed was produced in addition to the nephrotoxic tubular lesion by means of large doses of toxic agents. The effect on the vascular system in the kidney was directly observed at varying periods of the intoxication by the method of Schlegel (*2*) that we have previously mentioned.

A series of 65 animals were used in the visualization of the renal circulation, 20 as controls to establish the constancy of the normal pattern and 45 that were given the following well-known nephrotoxic poisons: uranyl nitrate, potassium bichromate, corrosive sublimate, carbon tetrachloride, sodium potassium tartrate and di-ethylene glycol.

The procedure of vascular visualization was the same in all experiments. The animal was placed under Dial anesthesia, the abdomen opened and then closed by clamps so that the kidneys could be removed in a matter of a few seconds. After a one-half hour interval, 1.0 cc. of 4 per cent thioflavin S solution per kilo was injected into the ear vein in a three second period, one renal artery clamped and the kidney removed at a measured time interval of six to eight seconds from the beginning of the injection. Free hand slices, both sagittal and longitudinal, were immersed in glycerin and observed with a binocular microscope under incident Wood's light at a magnification of 8 to 12 ×.

The pattern seen in the normal animal is remarkably constant (Plate 37A). All the glomeruli throughout the cortex are evenly and brilliantly illuminated and no dark glomeruli that did not receive the dye by way of the circulating blood are evident. The cortical background of tubules is faintly illumined by the natural fluorescence of the renal tissue and by the small amount of dye which has diffused out of the vessels. The intensity of the relative contrast between vessels and glomeruli and background tissue therefore depends on avoiding this diffusion; hence the need of speed in removing the kidney as soon as the blood has fully perfused the organ. It may be recalled that the circulation time in the rabbit is of the order of 7.0 seconds so that three seconds is ample time to accomplish the optimal visual effect.

On the faintly fluorescent background of the cortex are seen the brilliantly illuminated sections of the arterial tree as the chance cut of the razor has exposed them; large branches of the renal artery, "arcuate" arteries, and interlobulars with, in the fortunate section, appended delicate afferent vessels and glomeruli and, less clearly, the irregular plexus of intertubular capillaries and venules. In the medulla a brighter zone of primary tissue-fluorescence is present in which lie more brilliant efferent capillaries from the cortex. For a more detailed description of this functional-anatomical presentation of the renal circulation the reader is referred to the studies of Schlegel and Moses (*21*).[5]

The general appearance of the renal circulation at periods from 3½ to 70 hours after the administration of the poisons was relatively similar. All showed in varying degree the pattern of an irregular cortical ischemia.

Plate 37B shows the kidney 19 hours after the subcutaneous injection of 400 mg. per kilo of sodium potassium tartrate. There is an extreme ischemia with an irregular area to the left where considerable blood and dye have reached the glomeruli; in this area diffusion through the vessel walls is pronounced.

In Plate 38A is shown the vascular pattern 4½ hours after the intravenous injection of 24 mg. of corrosive sublimate per kilo. On the greyish, natural fluorescence of the renal cortex only a few scattered, irregularly illuminated glomeruli and cut branches of the smaller renal arteries are seen. The second kidney (38B) into which

[5] The pattern of the renal circulation displayed by this technique which depends solely on the functional forces that operate under physiological conditions is strikingly different from that produced by the artificial injection under pressure of various substances that hitherto have been the main source of our visual impressions of the nature of the renal circulation. No matter how carefully selected the injection material and how meticulously regulated the pressure, it is apparent that the distended, tortuously obese appearance of capillaries and glomeruli which forms the subconscious basis of our present concept of the renal circulatory bed is an artifact that is never simulated under living conditions.

blood was deflected by the clamping of the opposite renal artery and which therefore had received blood for a period three times as long as the first, thus allowing the accumulation of more dye in the vessel wall, shows more and brighter glomeruli and vessels but a comparison with the even pattern of the normal kidney (Plate 37A) which received dye over a lesser period, indicates that there is a persistence of irregular areas of cortical ischemia where no glomeruli are evident.

In Plate 39A, uranyl nitrate, 40 mg. intravenously per kilo after 70 hours, an irregular area of dark ischemia is seen in the center of the cortex. Plate 39B shows the kidney 19 hours after an intravenous injection of 30 mg. per kilo of potassium bichromate. The ischemia of the cortex is obvious.

Plate 40A shows the kidney removed on the second day after two subcutaneous injections of 4.0 cc. carbon tetrachloride; in Plate 40B the kidney after a 24 hour period in which the animal took *ad lib.* 5 per cent di-ethylene glycol in his drinking water. In both the irregular pattern of cortical ischemia is evident.

A visualization of the renal vessels at various periods after the administration and during the action of nephrotoxic agents leaves no doubt that over an extended time there is an irregularly distributed disturbance in blood flow through the renal cortex. A comparison of the resulting vascular pattern with that which Schlegel and Moses (21) have demonstrated to follow the injection of adrenalin, a form of renal ischemia concerning whose mechanism of origin we have more information, allows certain speculative interpretations in regard to the situation that obtains in the cortical ischemias following renal poisons.

As Schlegel and Moses have shown, and as might be expected from our pharmacological knowledge of its mode of action, the renal ischemia of adrenalin action is abrupt in onset, intense in degree and relatively short in duration. In combination with the apparent irregularity of its action on the terminal branches of the renal arterial bed, these factors produce temporarily patches of almost bloodless cortical tissue in which, at times, can be seen totally ischemic glomeruli. There is also an apparent decrease in blood flow through the medulla. Such is the picture that might be expected to follow a sudden, complete, but evanescent spasm of small arteries, presumably chiefly interlobulars and glomerulus afferents.

Judging from the appearance of the toxic ischemic vascular pattern it would seem that the ischemia, though equally irregular in its distribution, is less absolute; a few shrunken, *i.e.*, not fully irrigated, dimly illuminated glomeruli are scattered throughout the relatively bloodless regions giving an appearance that suggests that a mere trickle of dye-containing blood had been flowing in the involved areas. This impression is strengthened by the appearance of the second kidney, if it is removed and examined after a period of dye-containing blood flow two to three times as long as that which had occurred in the first kidney: more glomeruli and vessels are seen to be fluorescent, though there still is nothing comparable to the even, bright pattern of the normal animal. Evidently the dye can accumulate in the wall of the vessel with time even with a considerable reduction of blood flow. Another effect that results in a somewhat different picture of toxic cortical ischemia from that seen after the action of adrenalin is possibly due to damage to the vessel wall and an increased diffusion of the dye into the cortical tissue.

The similarities and differences between the two patterns of vascular disturbance show therefore that in the case of severe nephrotoxic poisoning there is a long-continued, irregularly distributed ischemia of the renal cortex, a potential cause, as the Van Slyke-Phillips experiments demonstrated, of irregularly scattered disruptive tubulorhextic lesions. Dissections of the nephrons from the kidneys of the animals of our experiments show that, besides the lesions in proximal convolutions due to the poison, the random disruptive lesions are in fact present.

There was considerable variation in the degree of damage noted in the kidneys from the individual experiments; in some the nephrotoxic lesion limited to the proximal convolution alone was evident and the remainder of the nephron was normal except that the lumens of the ascending limbs and distal convolutions were filled with epithelial debris and casts. The epithelium of these latter segments though compressed showed well preserved nuclei and even a normal mitochondrial pattern. In other animals, however, in which a well developed ischemic pattern had been

demonstrated in the renal cortex there were found typical tubulorhextic disruptions occurring in all parts of the nephron including the proximal convolutions.

In Plate 41 is seen a proximal convolution from rabbit 60 which had received 0.4 gm. of sodium potassium tartrate per kilo subcutaneously and which was killed 19 hours later. The ischemic pattern in this animal is shown in Plate 37B. It will be noted that in the first few coils of the convolution the nuclei may be faintly seen in the heavily stained cytoplasm of the intact epithelium; from this point onward the tubule is swollen and the necrotic epithelial cells have disintegrated into cellular debris. This lesion is that of typical nephrotoxic damage: at A, however, the basement membrane is ruptured and a short segment of tubule shows the tubulorhextic lesion. Similar disruptive lesions are found in the ascending limbs of Henle's loop and in the distal convolution. Plate 42A from another animal which received the same dosage and was killed on the second day shows an extreme form of the lesion, with dissolution of the basement membrane throughout and, in the right descending portion of the illustration, disruption.

In Plates 42B and 43A are shown distal convolutions and ascending limbs of two rabbits which had been given 40 mg. of uranyl nitrate per kilo intravenously and killed 70 hours later. The visualized ischemic pattern of the cortex of Plate 42B is shown in Plate 39A. The proximal convolutions of both these animals showed extensive nephrotoxic damage with the typical specific localization in their middle and terminal portions. It will be noticed that the disruption of the tubule wall involves only short segments in both examples and that there is no evidence of a diffuse nephrotoxic necrosis in the remaining portions.

Plate 42C and D are from a rabbit that was given 15 mg. per kilo of corrosive sublimate and killed 18 hours later. The pattern of cortical ischemia is seen in Plate 38A and B. The proximal convolutions showed typical nephrotoxic damage in the middle and terminal portions. The short segment of disruptive tubulorhexis at the junction of ascending limb and distal convolution is clearly evident, while in both preparations the normal appearance of the distal convolution indicates that there was no general toxic involvement of its epithelium.

The disruptive effect of ischemia occurring in di-ethylene glycol poisoning in rabbits which had taken *ad lib.* this toxic agent in 5 per cent solution in their drinking water for five and seven days is seen in Plate 43B and C and Plate 42E. Lesions of slight degree and moderate extent are shown in plates 42E and 43B; note the normal nuclei in both ascending limb and distal convolution except where the ischemic disruptive lesion has occurred. In Plate 43C there is extensive and extreme damage with dissolution of the basement membrane and destruction of epithelium throughout two greatly swollen ascending limbs and distal convolutions from another animal which had taken the poison for seven days. Disruption has occurred where the two distals are adherent and there is a large collection of black stained debris at this point. The patchy cortical ischemia of a similar animal is shown in Plate 40B. The nephrons of all these animals showed an extensive nephrotoxic necrosis in the terminal half of the proximal convolution, this portion being greatly swollen; even the typical vacuolated appearance of the necrotic epithelium is evident (Plate 44). There is no disruptive ischemic lesion in this proximal convolution but others showed irregularly scattered segments of tubulorhexis.

In Plate 43D the disruptive lesions associated with the cortical ischemia (Plate 40A) that followed 48 hours after subcutaneous injection of 4 cc. per kilo of carbon tetrachloride are illustrated. The areas of tubular damage are quite small and localized and complete disruption of the tubule wall has not resulted. The proximal convolutions showed a typical nephrotoxic necrosis of the epithelium of their terminal portion.

In summary, the lesions seen in the kidneys of the experimental animals poisoned with various nephrotoxic agents all showed the typical involvement of the proximal convolution that is characteristic of this group of substances. Figure 9 shows *camera lucida* tracings of nephrons with the localization of nephrotoxic damage from various poisons.[6] In other kidneys in which the

[6] It should be emphasized that the production of exact differences in localization of the lesion demands careful experimental control for their demonstration: if too large a dose is given the area of damage tends to spread

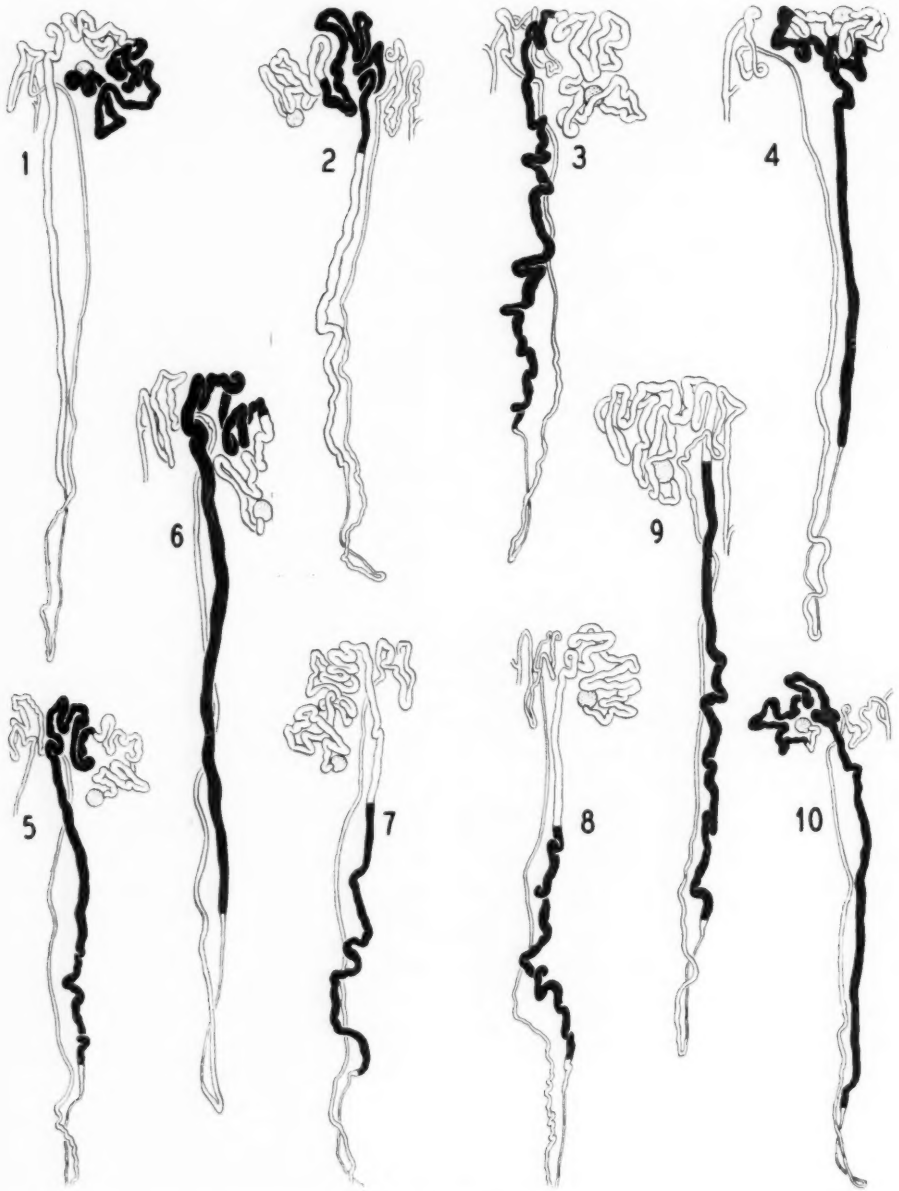


FIG. 9. LOCALIZATION OF NEPHROTOXIC DAMAGE IN THE PROXIMAL CONVOLUTION IN VARIOUS FORMS OF POISONING

1, Potassium bichromate; 2, uranyl nitrate; 3, corrosive sublimate; 4, sodium potassium tartrate; 5, potassium chlorate; 6, di-ethylene glycol; 7, carbon tetrachloride; 8, mushroom poisoning; 9, serine; 10, sulfonamides. The necrotizing effect of moderate doses is shown by black areas; with large doses the area of localization tends to spread and there is the further complication of an ischemuric episode with the development of disruptive tubular lesions. Magnification 20×.

Schlegel technique had demonstrated that there had existed an irregular and patchy cortical ischemia, the disruptive lesions of ischemic damage were found scattered at random throughout the length of the nephrons.

The experimental method, by its opportunities for control in dosage and consequent damage, allowed the demonstration of early forms of these ischemic lesions; Plate 42C, D and E and Plate 43B and D, for example, show short, limited areas of tubule swelling and basement membrane dissolution in stretches of tubule, the epithelial cells of which are generally normal. The similarity of these early lesions to those produced in the Van Slyke-Phillips experiments by purely ischemic insult in which no toxic substance was concerned (Plates 30 and 32) is striking evidence of a different manner of origin of the scattered tubulorhextic lesions from that of the nephrotoxic damage in the proximal convolutions. In our experimental procedure the only factor introduced to cause a disturbance in renal blood flow was the use of large doses of the toxic elements for this was adequate to establish the point of our argument. It seems certain, however, that if the other complicating factors, such as dehydration, vomiting, colitis and even "shock" that accompany fatal human poisoning, had been reproduced in the experiment, then the renal lesions in the animals would have departed as far from the classical description of the effects of toxic substances as did those of the human cases of poisoning which we have previously described.

along the course of the tubule until in the end the greater part or all of the proximal convolution is affected and the selectivity of the toxic action is lost. There is also, as we have just shown, the complication of renal ischemic damage that accompanies the graver intoxications and this may occur in the proximal convolution as well as elsewhere and so disturb the pattern of the specific lesion. The selective localization of the action of various toxic agents, as observed in the experimental animal, does not therefore, as we have seen in our dissections, commonly occur in human poisoning, but it has nevertheless considerable theoretical importance in regard to the recognition of differences in the seat of functional activities in the proximal convolution (29). The only confirmations by dissection of Susuki's original conclusions, which were drawn from the study of histological sections with which we are familiar, are in the case of uranium (28) and corrosive sublimate (15). During past years a considerable number of other poisons have been studied in this laboratory and these findings are shown in Figure 9. This is not a diagrammatic, *i.e.*, interpretive presentation, but shows *camera lucida* outline tracings of actual nephrons with the area of nephrotoxic damage indicated by cross hatching.

SUMMARY, REITERATION, AND SPECULATION

It is clear that what has been revealed by this investigation, both in its examination of the structural status of the human kidneys and by its experimental procedures, does not lead to a confirmation of the currently prevailing concept of a "kidney disease" the "unique" pathological characteristics of which can be described as "lower nephron nephrosis," for the lesions in the kidney are not peculiar to or localized in any portion of the nephron and their diverse character would seem to strain even the meaningless embrace of that most tolerant of terms, "nephrosis."[7] Nor are our findings compatible with the present theories of this curious malady, the pathogenesis

[7] The term "haemoglobinuric nephrosis" is equally unsatisfactory, for it can hardly be used to apply to the whole group (Acute Renal Failure associated with traumatic and toxic injury) since in many examples haemoglobin is not concerned at all in the lesion. Even in those in which heme pigments are found either in casts or in the renal cells it is doubtful that they play any part as a "toxic factor" in the production of the characteristic tubular lesions. We have shown that the cells of the tubule are well preserved in the presence of deposits of heme pigments either free or in casts and that it is only coincidentally that lesions and pigments are found together. Extensive experimental evidence indicates that in any moderate amount haemoglobin does not damage the nephron: it is in fact used experimentally as a measure of glomerular permeability and does not sufficiently disturb renal function to interfere with the use of clearance methods. Its appearance in the cells of the proximal convolution in droplet form is a special case of the normal activity of these cells in absorbing protein from the tubule fluid and digesting it (9). In the case of haemoglobin this metabolic activity is indicated by the appearance of free Fe in the renal cells. It is true that, as in all digestive processes, an over-loading of the mechanisms can produce indigestion with resulting transient ill effects on the digestive organs, in this case the renal cells. A similar indigestion of serum proteins with the development of cellular abnormalities, hyaline droplets, occurs in the nephrotic syndrome of chronic glomerular nephritis, but these tubular alterations are entirely different in character from those which we have been considering. It is also true that too-bountifully a good meal has its ill effects, but one would hesitate to designate this not unpleasant temporary discomfort as a "disease" of the stomach.

of which fits no functional or anatomical pattern known to the kidney and whose "cause" is sought in toxic factors such as heme pigments or among less determinate entities such as "products of traumatized tissue," "electrolyte imbalance" and even "allergies."

And yet amidst all this elaboration of hypothesis and semantic confusion the syndrome does stand clearly outlined as the most important of the acute disturbances of the urinary system. This place it maintains not only by the frequency of its occurrence and the gravity of its ultimate effects, but more importantly by the remarkably protean aspect of its origin: it would seem that almost any clinical situation—trauma, malarial infection, obstetrical accident, transfusion reaction, postoperative complication or intoxication—may end with its manifestations of renal failure. There must be, therefore, some pathogenetic unity behind this screen of functional, pathological and clinical diversity.

Dr. Homer Smith solves the nosological puzzle by bringing all Acute Renal Failure together in Chapter XXIV of his recent monograph (1), and it is evident that from the functional viewpoint a most reasonable unity results. But the question may arise if the job has been completely finished, for a morphologist will be disturbed to note that the chapter begins with a consideration of the kidney in sublimate poisoning and ends with the "crush kidney," and from what he knows of the structural changes that occur in these two conditions, the squeezing of any two such "entities" into one pigeon-hole may seem a unification of diversity attained only by considerable Procrustean violence. As he sees quite different structural effects, he will suspect different pathogenetic causes; the sublimate kidney seems obviously the result of a toxic factor while the functional data stress the importance of some disturbance of renal blood flow in the "crush kidney." Though in the last analysis toxic and ischemic damage may, as interferences with cellular respiration, be similar, at the more immediately accessible level of their mechanisms they seem quite different, a difference indeed that might determine such very practical matters as prognosis and manner of treatment. And so a morphologist justifiably might be doubly perplexed to find not only one but both of his conceptual progeny in this galley of Acute Renal Failure.

Functionally the two examples of the renal lesion are similar, for clearly the kidneys of both sorts have "failed," but this is not enough. Structurally they are different; again apparently the all-too frequent dichotomy of the functional and structural approach. Believing that the best way to solve a dilemma is to avoid it, we suggest, at the hazard of considerable reiteration, that the situation be reviewed from the beginning and, quite naturally, that the start be made from the structural standpoint.

The common structural lesion in all members of the group of Acute Renal Failure is tubular damage, and if a name be required, they may all be said to be examples of a "tubular nephropathy." This is not to assume that the glomeruli are entirely normal; proteinuria and consequent cast formation are clear evidence that the filters are leaking, an abnormality which must be structural in mechanisms which are invisible to present methods of observation. For this practical reason only we suggest the use of the tubular aspect of the renal lesions as the "structural characteristic" of the pathological entity.

When the tubules of the nephrons from a large number of examples of this group of varied clinical origin are examined by a method which adequately displays not only the cytological lesion but the topographical continuity of the nephron, it becomes apparent that there are two quite different sorts of tubular damage.

One sort of tubular damage is the well-known nephrotoxic lesion that results when some poison in the blood stream is distributed evenly and equally to every nephron of the two kidneys. Like many foreign substances, it is filtered through the glomerulus and absorbed by the cells of every proximal convolution. A considerable intracellular concentration above what exists in the circulating blood is thereby established and the renal cells die. The basement membrane is not visibly affected in this lesion nor are the epithelial cells of the other portions of the nephron; both escape because in regard to the toxic substances they are functionless. The tubule lumen below the site of actual damage may be filled with the products of both the glomerular lesion, that is, coagulated protein in the form of casts, and epithelial destruction.

The other sort of tubular lesion, first recognized by Dunn, Gillespie and Niven in the "crush kidney" and thought by many to be peculiar to the "lower nephron," is in almost every way the antithesis of the nephrotoxic tubular lesion. Its distribution within the kidneys is entirely random; some nephrons, often in groups, are involved while others are free of it. Within the nephron any portion may be the seat of damage, from the origin of the tubule at the glomerulus to the collecting tubule. One of the most frequent sites is the second half of the proximal convolution which extends into the outer medulla and in histological section is easily confused with the ascending distal limb of Henle's loop.

The lesions themselves differ strikingly in their appearance from what is seen in the nephrotoxic lesion. Rarely is any whole segment of the nephron affected; there may be only a short stretch of tubule or a series of isolated spots that lie distributed over several anatomically and functionally different divisions of the nephron. In these areas the entire tubule wall is affected, including the basement membrane, which may be broken with its remnants still visible or thinned to a barely perceptible sheet or completely disintegrated. The epithelium within it is dead and desquamated so that the result is a disruption of the continuity of the tubule. From this characteristic the term "disruptive" or "tubulorhextic" has been suggested to distinguish it from the nephrotoxic tubular lesion.

If casts are present at the site of the disruptive lesion, and they often are not, they may extrude from the gaping hole in the tubule. In the intertubular connective tissue in the vicinity of the rupture there commonly occurs an inflammatory reaction with leucocytic infiltration and the formation of inflammatory granulation tissue which extends by growth into the lumen of the disrupted tubule; if larger vessels are present "tubulo-venous anastomoses" may form.

If one wishes to speculate as to the origin and cause of a lesion of this sort on the basis of the purely morphological data, a procedure that was necessary in the earlier stages of this investigation, it certainly is quite clear from what is known of the functional and anatomical pattern of the kidney that no such damage can conceivably be the result of a poison reaching the nephrons by the blood stream. This conclusion disposes of a vast amount of speculative hypothetical possibility, such as an origin resulting from "toxic products of tissue damage." A great deal more will have to be said about the part that toxic damage may play in the group of Acute Renal Failure, it is true, but in our immediate and restricted problem, the cause of the disruptive tubular lesion, we can be reasonably satisfied that this element is eliminated.

Another considerable simplification in the decision as to possible causal factors results from the observation of casts, pigment and debris deposits in the continuity of the dissected nephrons for these objects have a much greater extent and complexity than is suggested by their appearance in histological sections. It is readily seen in the dissected nephrons, for example, that extensive deposits of extraordinary length of heme pigment and thick casts, either containing or free of heme pigment, may distend ascending limbs, distal convolutions and connecting tubules and yet a completely intact tubular wall persist in which both the renal epithelial cells and basement membrane, though thinned perhaps by tension, are not otherwise damaged. Moreover casts and pigment deposits are extremely uncommon in the proximal convolution where disruption is frequent. In some instances it is true that casts or pigment deposits coincide with tubular disruption, but the more frequent negative evidence precludes any possibility of a causal relation.

There remains as a causal factor to be considered, the phenomenon of reduced renal blood flow which, as Dr. Smith's chapter emphasizes, accompanies so many of the renal disturbances of this group. This factor would be equally incredible if the current concept of a lesion unique in its localization to the lower nephron were true, but a morphologist would be quick to seize upon the factor of renal ischemia as a likely cause of scattered and random lesions in the kidney for he has seen many such examples of cause and effect in other tissues and organs. Moreover this conclusion from his general experience is made more certain by the visualization of the mechanisms and patterns of renal ischemia by Schlegel and Moses whose technique allows an objective and exact morphological view of the exact status of the renal

blood flow at any given moment and under any particular condition.

One of the most important contributions of this technique is the demonstration of the remarkable patchiness of renal ischemia; in the experimental conditions which we have examined there is rarely an over-all, even reduction of flow through the vascular bed; some areas are bloodless, others appear congested. In viewing this total pattern one might wonder if there had been much if any reduction in total renal blood flow but rather if there had not occurred only an irregular distribution of blood flow within the cortical tissue.[8] The implications of this possibility are of considerable importance both to the morphologist and the functionalist. To the former it explains the peculiar random distribution of the lesions he sees in the nephrons and to the latter it may bring further complications in his interpretation of the significance of "clearances" in the pathological kidney for it would appear that some nephrons or even parts of nephrons can be bloodless while others are irrigated with an excess and yet the total blood flow may be approximately normal. In uranium poisoning, for example, clearance methods indicate a normal renal blood flow (1), yet Plate 39A shows a definite, irregular cortical ischemia.

All this preliminary spade work of *a priori* evidence and speculation as to the part that might be played by renal ischemia in the production of disruptive tubular lesions may well seem to have been a labour of supererogation when we come at last to the actuality of its experimental production by Van Slyke, Phillips and their co-workers. In anatomical material from delicately balanced experiments where the operative margin between too much and too little must have been peculiarly precarious, our dissections have shown that they produced the typical disruptive lesions both by direct ischemia following clamping the renal artery of dogs and by that which follows reflex renal vasomotor constriction associated with muscular trauma. This demonstration, with the extensive functional evidence which they have produced, would seem to settle the question as to what is the cause of the disruptive tubular lesion in the kidney of shock. Besides this, the chief contribution of their experiments, there are certain subsidiary evidences of considerable value.

For example, many attempts have been made to reproduce the "crush kidney" by clamping the renal artery with dubious success. The Van Slyke-Phillips kidneys show clearly in the large series of their experiments every degree of renal damage from what might be called an infarction of the kidney to the typical, scattered lesions of disruptive tubular damage in isolated nephrons. Exactly the right degree, both in duration and extent, of renal ischemia is required to produce the distinctive, disruptive lesions of the shock kidney. Moreover earlier investigators were discouraged in their attempts by finding involvement of the proximal convolution when the key lesion they were seeking was presumably confined to the "lower nephron." They could not reproduce the "unique" lesion which does not and, from the structural and functional pattern of the kidney, could not exist.

Another finding of considerable importance in the experimental material was the observation that in tubules containing well developed disruptive lesions as a result of the renal ischemia that followed extensive traumatization of muscle and where heme pigments were excreted into the tubule lumen, absorbed by the epithelium of the proximal convolution and also deposited unchanged in cast form in the distal convolution, there was no structural evidence of toxic injury to the renal cells, even to those which were contiguous to the points of tubular disruption.

The most sensitive morphological criterion of toxic damage to the renal epithelium requires im-

[8] In the cortical ischemia following the administration of toxic substances we have never seen a pattern suggesting a deflection or shunt of blood from an anemic cortex to the medulla. In this regard, *i.e.*, the production of the Truetta phenomenon, it might be said in passing that the normal anatomical configuration of the circulatory bed in the kidney is such that any drop in the pressure head in the larger distributing arteries (interlobar or arcuate) will automatically result in a cessation of flow at the periphery of the system, *i.e.*, the terminal branches of the interlobular arteries and so produce a zone of sub-capsular ischemia which, as the pressure head further falls, will spread downwards toward the medulla.

The medulla may thus continue to receive blood from the efferents of the juxta-medullary glomeruli at a time when the cortex is largely ischemic. Such a maldistribution of blood is not a shunt, but only the final stage in the persistance of the failing circulation.

mediate and perfect fixation of the tissues and so it cannot be applied to human autopsy material. This evidence of a toxic lesion consists in a dissolution of the mitochondrial rodlets of renal cells, yet in the experimental material these structures were found well preserved throughout proximal convoluted tubules that contained extensive disruptive lesions of ischemia. To the morphologist this seems proof that neither products of the muscle injury nor the heme pigments could have had even a minimal toxic effect on the tubules of these experimental "crush" kidneys.

Though such refined cytological evidence is unavailable in human kidneys from autopsy, the general tenor of the argument would seem to indicate with reasonable certitude the pathogenesis of one member of the group of Acute Renal Failure, namely the "crush kidney" of traumatic injury: the structural lesion is characterized by the disruptive tubular type of damage with no nephrotoxic tubular damage and renal ischemia concomitant with or a sequel to the syndrome of circulatory collapse or general shock is the reason for its occurrence.[9]

To return now to Dr. Smith's category of Acute Renal Failure, it is found that the "crush kidney" comes at the end of a series, the first of which is the renal disturbance produced by uranium poisoning; between the two are distributed functional disturbances that result from the most varied of clinical conditions. Regarded purely on the basis of their structural status no more apposite serial arrangement of the members of the group could be made by the morphologist, for the uranium kidney in its simpler form seems far removed from the "crush kidney," its tubular lesions being by classic description of the nephrotoxic sort with no evidence of involvement of anything but the proximal convolutions and with no disruptive tubular damage.

When two structural lesions are antithetical it might be contended that they can hardly be compatible members of the same pathogenic group and that at last the much desired correlation of our structural-functional data has broken down. But on considered thought and in the light of what we have seen in the sublimate kidney of man, the discrepancy is not so real as would appear and it arises only because two very different circumstances of renal insult have been compared, namely one that arises spontaneously under stress in man and another that is produced experimentally in the laboratory animal under carefully controlled conditions.

When a man is poisoned in the course of natural circumstance, by corrosive sublimate for example, in a dosage of uncontrolled amount and passes through periods of excitement and depression, of vomiting and dehydration, of violent enteritis and exhausting diarrhea that end in a state of circulatory collapse, what has occurred to him as a living organism is very different from what has happened in a rabbit which has been given moderate intravenous injection of the same substance and which passes quietly and ostensibly without clinical symptoms or disturbances into renal failure. It is therefore not surprising that the lesions in the human kidneys are not examples of simple nephrotoxic damage, but that there are also present, as evidences of circulatory disturbances, the disruptive tubular lesions of ischemia. We have shown that this is also true in the kidneys of rabbits that have received large doses of nephrotoxic poisons and have demonstrated by visualization of the renal circulation that during the course of these severe intoxications periods of renal ischemia occur which might reasonably account for the mixed nature of the tubular lesions.

To put the varied possibilities in much oversimplified case form, an individual may therefore suffer Acute Renal Failure under the following circumstances:

1. Crushing injury—shock—renal ischemia—random disruptive tubular damage—anuria—uremia—death.

2. Anti-freeze or sublimate intoxication—diffuse nephrotoxic proximal damage—dehydration, K poisoning, enteritis, diarrhea—circulatory collapse—renal ischemia—random disruptive tubular lesions—anuria—uremia—death.

3. Moderate poisoning—diffuse nephrotoxic proximal damage—oliguria or temporary anuria—regeneration—recovery.

This last group is obviously a theoretical instance for the pathologist for even in the classical

[9] In the crush syndrome of man it is of course conceivable that the vasomotor spasm which is the "cause" of the ischemia might be the result of a poison acting on the arteries and arterioles. The "cause" of the epithelial lesion under these hypothetical conditions would remain, however, the ischemia.

and "purest" example of nephrotoxic damage, the sublimate kidney, all fatal cases showed the effects of ischemia by the presence of disruptive tubular damage. It is one, however, which he can accept by analogy from his experimental experience and that of his clinical confreres.

By this concept unity of pathogenesis is obtained in both the structural and functional aspects of a renal syndrome that seems at first glance to be a bewildering clinical congeries of placental separations, transfusion accidents, traumatic or surgical shock of varied origin, malarial infections and diverse intoxications from "anti-freeze" indulgence to sulfonamide therapy.

The unifying element in this heterogeneous complex, Acute Renal Failure, is renal ischemia, for under whatever clinical circumstance renal ischemia may develop, there the disruptive tubular lesions, either alone or mixed with the effect of antecedent toxic damage are found along with the functional disturbances of oliguria or anuria and all the consequent varied tubular dysfunctions that are revealed by clearance techniques. The syndrome remains, therefore, on the firm basis of an adequate correlation of its structural and functional characteristics an entity.

This unity, however, arises not from what happens primarily in the kidney but outside it in that area so susceptible of disturbance, the general circulation. What confronts us is therefore not a "renal disease" but a *circulatory episode* that may complicate any clinical situation and we therefore suggest that it be so recognized under some appropriate term.

In various clinical situations from the shock of crush injury to chemical intoxication, one may, on the development of this clinical-pathological syndrome, speak of the occurrence of an "ischemuric renal episode," thus emphasizing in a sort of portmanteau word the principal phenomenon of cause and effect, namely the renal ischemia and, to use an older term, the ischuria.

The structural status of the kidney in the case needs, or perhaps one might say deserves, no specific pathological designation, any more, for example, than the structural status of the kidney in chronic passive congestion is given a pathological-anatomical name.

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With the unity of pathogenesis of the syndrome established and an appropriate term for its designation suggested, we can now turn to various problems regarding the correlation of the structural lesions with their functional and clinical manifestations. Perhaps the first of these that presents itself is the "cause" of the oliguria or anuria which is closely related to all those other more recondite disturbances that are revealed by clearance methods. It is surely too much to hope that they can all be elucidated, but it is encouraging to see that the earlier dichotomy of interpretation is progressively narrowing as both functionalist and morphologist gain in experience of the renal lesions and, it might be added, of each others' ideas.

From the morphological viewpoint it seems certain that there is no single "cause" of oliguria but a great collocation of them, or as we would prefer to put it, that a constellation or "field" of structural phenomena determines and at the same time is a consequent of the functional disturbances. This latter form of causal concept is particularly useful, because as will be evident in our listing of them, the "causes" are interdependent, one modifying, often intensifying and at times nullifying the other. Moreover, it is often impossible to say whether a certain structural change is the "cause" of a certain functional phenomenon or whether the latter is not the determinant of the former, for "structure" and "function" are not two separate categories of being but different aspects of the same thing (25). The morphologist who has no way of measuring quantitatively the many effects of either aspect of the lesion should, however, call attention to them, leaving the final decision of their relative importance or priority for future decision.

There can be little doubt that renal ischemia may result in decreased filtration in the earlier stages of the development of the syndrome. There is small likelihood, however, that the simplicity of this factor remains long uncomplicated. First of all, the visualization by the Schlegel technique of the patchy cortical ischemia which produces the tubular lesions is quite a different pattern than what might be suggested by the simple statement that cortical renal blood flow is reduced. As we have suggested, perhaps over-all renal blood flow is not greatly decreased and yet the patchy maldistribution of blood to nephrons

may still result in tubular dysfunction and eventual structural damage. In any case the tubules under conditions of ischemia must suffer functional derangement and presumably one of the first and least effects, occurring long before the development of a structural lesion, would be a reduction in water absorption. At least this sequence of events is demonstrable in the perfused kidney of the frog (30), and so to the factor of reduced filtration is added a counter factor.

It would not seem likely that this period of the slightest of tubular dysfunctions can long endure; with the beginning of structural change an increased permeability of the tubule wall and back-diffusion begins. The methods of clearance have given much data at least for speculation in regard to the diffusion of the various substances through the tubule wall and the morphologist cannot be very helpful in the interpretation of this complex problem. He can, however, emphasize the importance of a much simpler form of tubule leakage to which functionalists have paid less attention, and this is the gross pouring of tubular fluid *in toto* through holes in disrupted tubules at every level of the nephron from the origin of the proximal convolution to the collecting tubule.

The leakage of tubule fluid into the interstitial tissue, a factor that might directly reduce the amount of fluid delivered to the collecting system, would seem to be one element in the production of the intertubular edema which may at times be so apparent in histological section. This edema in turn seems an important factor in the production of a rise in intrarenal pressure, a potential cause of oliguria by its pressure on tubules with a direct impedance of flow and on vessels, with an indirect effect in the same sense by its compression of thin afferent capillaries and a consequent exaggeration of renal ischemia and lessened filtration.

Moreover, the flooding of the interstitial tissue with tubule fluid produces much more than an increase in physical tension. In many instances, following the administration of sulfonamides, for example, leaking fluid is an irritant which produces an exudative inflammatory reaction which becomes granulomatous in its later development. The degree of this "pyelonephritis" varies remarkably in different kidneys for reasons that are not always apparent. Perhaps one constant factor in its production is the protein which the escaping tubule fluid contains, this derived from the increased permeability of glomerular membranes as a result of the original ischemic damage. And so another modification of another sort of intrarenal tension, the oncotic pressure of the interstitial fluid, results with effects on tubule resorption and blood flow that are as yet not clearly understood.

The interminable discussion and argument in the literature concerning the part played by stoppage of renal tubules with casts may well be the result of a confusion between the "casts" that are seen in the urine as discrete plugs and the accumulations of epithelial debris, pigment, and proteins in every degree of sol-gel transformation that occur in the tubule of the nephron. Much of this confusion would be avoided if it were remembered that, judging from the results of microdissection, it is very improbable that any cast seen in the urine could have come from a nephron: urinary casts arise in the straight collecting tubules from which they are readily expelled. It is hardly necessary to state that any physical obstruction whether in the form of solid matter or an increased viscosity of a semi-coagulated protein fluid will lessen tubular flow; or that a lessening of flow for this or another reason will tend to exaggerate accumulation of these impeding materials, which in their turn will further decrease flow. Moreover any impedance in flow through the lumen of a ruptured tubule, or one even more permeable than normal, will increase leakage into the interstitial tissue and so will result in alterations of physical and oncotic intrarenal pressure. Again a circular effect on tubule fluid flow results extending beyond its own immediate viscious circle, and by the consequent disturbance of renal blood flow enmeshing, wheels within wheels, one cycle of oliguria-producing phenomena with another.

If we have over-laboured in what is admittedly a highly speculative manner the discussion of the question, "what is the cause of anuria," it is because one so frequently hears and even reads, where more considered treatment might be expected, argument brought either for or against some particular factor which doubtless seems to its proponent the definite, specific and therefore logically valid "cause." A great deal of such para-logical discussion we believe originates in the

subconscious use of a "linear" concept of causality and would never arise if the constellational or field concept were applied to the elucidation of what must surely be an infinite collocation of related phenomena among which we can at best define only a few.[10]

A few other clinical correlations may be briefly attempted. During the stage of recovery the volume of urine increases, at times rather suddenly, to a degree that might be considered a state of diuresis. In its explanation a morphologist can do little but draw on an imagination reasonably controlled by what he knows of the altered structural status of the kidney; certainly it is difficult for him to believe that any profound structural change has abruptly occurred in the damaged kidney, such as a sudden flushing out of impeding casts and debris from tubule lumens, for these structures have too much the look of permanency in his dissected specimens. What seems more likely to him is that a partial recovery of both blood flow and tubule function has occurred to that level where a moderate filtration is combined with a still inadequate tubular absorption of water, a situation mentioned earlier as a theoretical step in the progressive development of the functional lesion of oliguria. Under such conditions a very little filtrate from a few nephrons might result in a large volume of urine and such a change, being determined by increased blood flow, might develop suddenly as it depends on no alteration of the structural basis of the renal lesion but rather on a relaxation of vascular spasm.

To speak of reparative processes raises the question of the very considerable practical importance in problems of prognosis, prophylaxis and of treatment, of what may have seemed to be largely an excessive refinement, namely the sharp distinction we have made between the two sorts of tubular damage. As we have shown, the repair of extensive and severe disruptive tubulorhextic lesions is difficult of achievement; extensive but futile "regeneration of tubular epithelium" may occur with nevertheless a failure of reconstitution of nephrons whose structure is compatible with function. Nephrons so affected are probably lost forever and a return to adequate renal function must therefore come from compensatory processes in those that escaped the original ischemic insult. The number of these is doubtless determined in the early stages of the syndrome, so that the sooner renal blood flow is increased the better the chance of ultimate recovery. After the ischemuric episode has occurred and its disruptive lesions are established it would seem doubtful if any amount of renal circulatory improvement could do much good. The clinical instance of glycol intoxication which we have described, where after three weeks there had been no return to functional normality even with a reestablished "urinary output," is a good example. This is not true of those kidneys where the nephrotoxic tubular lesions predominate; here the intact basement membrane provides the scaffolding for epithelial regeneration and nephron reconstitution; if the individual can be maintained a sufficient time, recovery of a functioning kidney can occur although the great majority of the nephrons were originally damaged. Unfortunately, as our cases of glycol and sublimate poisoning illustrated, severe intoxication leads to the complication of a renal ischemia with added disruptive tubular damage, so that the prognosis in such instances is perhaps not better or may be even worse than in the simpler case of the "crush kidney."

Complication by an ischemuric episode with its disruptive tubular damage in cases of poisoning may well be a reason for the failure of such therapeutic agents as BAL in certain specific forms of poisoning and in any example of severe intoxication; no matter how efficacious this procedure may be in its effect on the nephrotoxic action of the poison, it seems clear that its mechanisms cannot obviate or relieve the effect of the complicating renal ischemia and its consequent disruptive tubular damage. A reference to the

[10] In this last regard, that is, our doubtless long to continue ignorance of the totality of renal fundamentals, it is well to remember that in the present problem, the explanation of a change in rate of flow through the renal tubules, we do not yet know how fluid can flow through the tubule even under the presumably relative simpler conditions that obtain in the normal nephron! Lichtwitz (31) was forced to the assumption that tubule fluid, like mercury in contact with glass, does not wet the wall of the tubule and Winton (32) after considerable calculation leaves the mystery with the pertinent suggestion that, in view of our uncertainty regarding this basic datum, we need feel no surprise if our explanations of its infinitely more complicated derivative problems prove inadequate.

case of sublimate poisoning treated with this agent will emphasize this point.

It may seem to some that a defect in our final conclusions is our inability, or disinclination, to give a specific anatomical-pathological name to the abnormal kidney in Acute Renal Failure. We believe, however, that the situation in the kidney, even from the relatively simpler aspect of its structural alteration, is much too complex to be covered by any meaningful term. A recent attempt to do so illustrates this difficulty: the conditions with which we are concerned are distributed variously among three categories, Upper Nephron Nephrosis, Distal Nephron Nephrosis and, apparently to catch the anomalous stragglers, Upper *and* Lower Nephron Nephrosis. It would seem unlikely that any sort of renal lesion could escape this nosological net, but what does one distinguish by the use of such all-inclusive terms? Only that there seems to be no Middle Nephron Nephrosis.

The structural lesions, if not the sort of "renal disease," that occur in Acute Renal Failure can, however, be accurately and meaningfully stated: they are: "nephrotoxic tubular necrosis" and disruptive tubular damage or "tubulorhexis," each with its distinctive structural characteristics both in regard to the sort of damage that is done to the tubule and to the location of the lesions. Of perhaps more importance to the clinician, each has its differential aetiological characteristic, a renal poison in the one case, and in the other the ischemia of the ischemuric episode. All these essential connotations are clouded or even completely obscured when an attempt is made to find a phrase that covers everything and describes nothing.

It is, therefore, when one attempts to pass from the objective level of the renal lesions to the higher abstraction of a "renal disease" that semantic difficulties with all their conceptual confusion result. The answer, of course, is not to take this unnecessary step, for Acute Renal Failure is the result not of a specific sort of altered kidney that can be given a name, but of the summation of various structural-functional lesions in several million nephrons (19).

It is quite true that under controlled experimental conditions Acute Renal Failure can be produced in two specific forms, one characterized by purely nephrotoxic tubular lesions limited to the proximal convolution or on the other hand, as our dissections of the material of Van Slyke, Phillips and their co-workers showed, a contrasting renal status with only disruptive tubulorhextic lesions so pure in form that even the mitochondrial elements of the intact tubule showed no evidence of toxic effect.

It seems very doubtful, however, if the clear-cut distinctions of the experimental situation ever occur spontaneously in Acute Renal Failure as it happens in man. The "crush kidney," or the kidney from individuals where an ischemuric episode has occurred during surgical shock, may approach the ideal example of purely tubulorhextic damage, but in the protracted course of the renal failure it will be the rare case where some complicating nephrotoxic damage to the renal tubules, such as the "cloudy swelling," has not occurred.

In the fatal case of Acute Renal Failure where an ischemuric episode has complicated poisoning, the abnormal structural renal status is from its origin always confused; there are both nephrotoxic tubular lesions peculiar to the specific poison in the proximal convolution and the disruptive tubular lesions of renal ischemia everywhere in the nephron, all so mixed and blended that their separate identities are at times difficult to distinguish even in the continuity of a single isolated nephron. Moreover, besides necrosis of tubules there are present the interstitial inflammatory reaction that follows tubular disruption and the abnormalities in the epithelial regenerative processes with the failure of restitution of functioning nephrons.

From the standpoint of pathogenesis, one phenomenon stands clear in this confusion of various tubular necroses, inflammatory reactions and regenerative abnormalities—the ischemuric episode—for the occurrence or not of this event determines the course and direction of the reactions in the kidney to the ill effects of traumatic and toxic injury.

CONCLUSIONS

In Acute Renal Failure related to toxic and traumatic injuries the structural alterations which are correlated with and which may reasonably explain the functional disturbances in the kidney are those of a tubular nephropathy.

Two sorts of tubule injury are encountered. The first is a nephrotoxic necrosis limited to that part of the nephron, the proximal convolution, which is functionally concerned with the handling of the poison. Since poisons are distributed by the renal circulation all nephrons are equally involved. The second type of lesion is a disruption of the renal tubule (tubulorhexis) due to focal cortical ischemia. It occurs at random among nephrons and in any part of a nephron.

In the kidney of any case of fatal Acute Renal Failure arising under various clinical circumstances these two lesions appear in varying proportions depending on the nature of the renal insult, whether toxic or circulatory or both. In Acute Renal Failure of non-toxic origin (shock, crush injury, etc.) the insult is purely circulatory, a part of or an episode in the general circulatory disturbance which has involved the peculiarly susceptible area of the renal blood flow. The term *ischemuric episode* is suggested to designate this complication.

Such a renal circulatory episode also may occur as a consequent of the general circulatory disturbances of grave intoxication. From this coincidence stems the unity of pathogenesis in the group of Acute Renal Failure as a whole the members of which include such clinically disparate origins as crush injury, transfusion reactions, obstetrical accidents and fatal poisonings.

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Plate 1

This figure shows the detail that can be seen in a normal nephron (rabbit) when fixed immediately in 10 per cent formalin, dissected after maceration in concentrated HCl and stained with iron haemotoxylin, mounted in H_2O without the use of any clearing agent and photographed through the thickness of the specimen (60 μ) at a magnification of 100 ×.

The nuclei are unstained and therefore appear as clear spots on the dark mitochondrial pattern of the renal epithelium. The decreasing gradient of the mitochondrial pattern is well shown, so dense in its first portion as to almost obscure the nuclei and fading almost to disappearance in the terminal medullary portion.

Certain discrepancies may be noted in the size of the objects photographed in the following plates. This is due to the pressure of the cover slip which flattens and spreads the glomeruli, actually 0.20 mm. in diameter, so that they appear out of proportion to less compressed tubules.

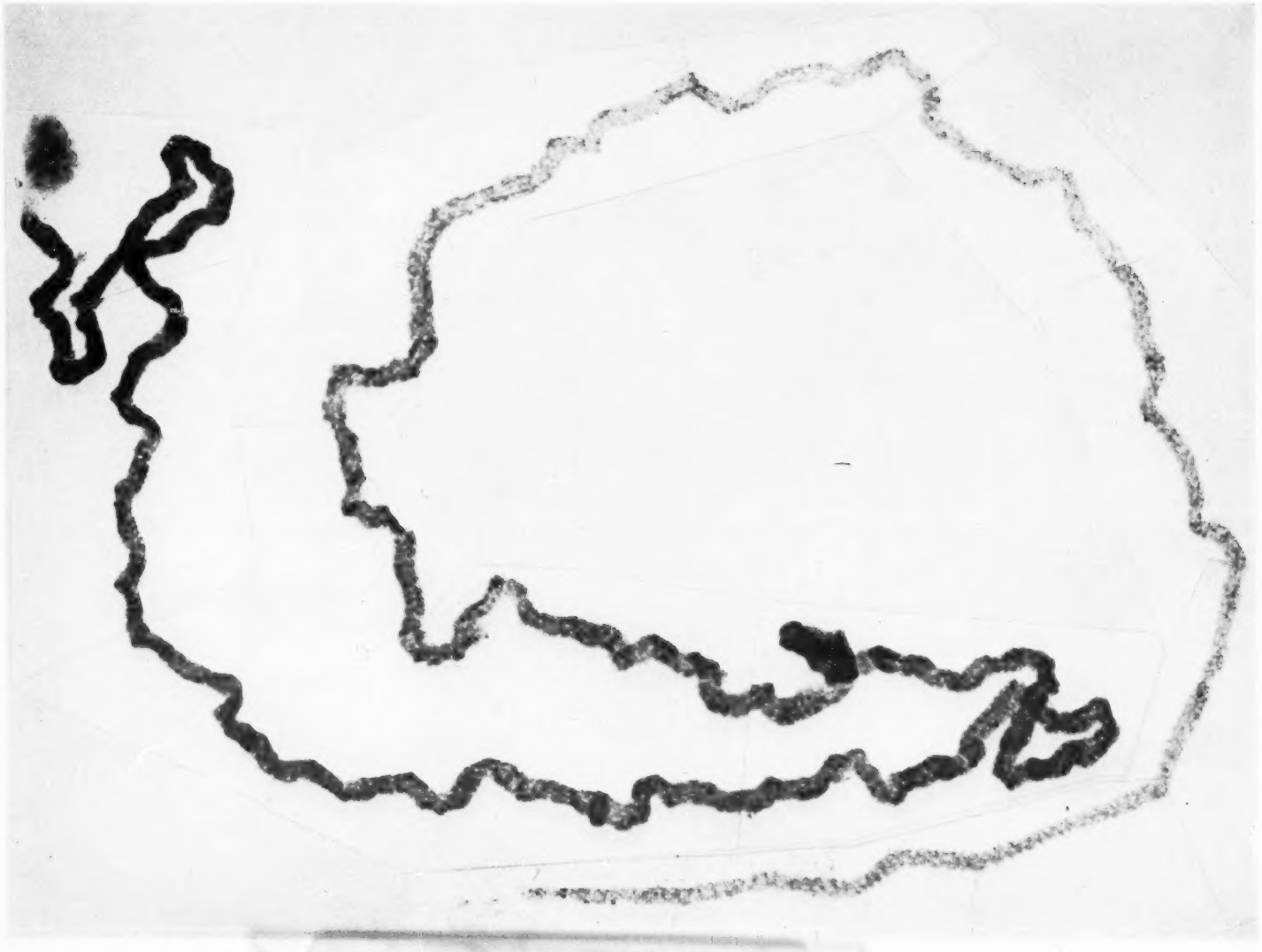


PLATE 2. DETAILS OF THE PRECEDING CONVOLUTION OF PLATE 1 AT A MAGNIFICATION OF 300×. (SEE LEGEND TO PLATE 1)

In A to E are portions of the convolution showing the decreasing gradient of the mitochondrial apparatus, so dense near the glomerulus as to obscure the nuclei (A) and practically invisible in the medullary portions (D) and (E). In favorable regions of focus and where not too densely packed together the basal ends of the rodlets can be seen at the arrows. In the mid-portion of the convolution where the mitochondria are less dense the brush border can be seen (C). The ascending limb is shown in F and in G the distal convolution with its stout heavy rodlets. In H is the transition of the proximal convolution into the thin, descending limb of Henle's loop. The tubule is over-stained to show the islands of cells in the proximal convolution as they progressively fade into the capillary-like thin segment. The diverticulum on the proximal convolution (A) is the only one we have ever noted in any species in this situation; they are common in the distal convolution of man (Plate 27).

The appearances noted above are only perfectly seen in immediately fixed material: 20 minutes delay at room temperature will cause a dissolution of the mitochondrial rodlets to a diffuse granular background. As in the histological section, human autopsy material therefore never shows the perfect picture of these finer structures; this contrast will be apparent in a comparison of the human with the experimental material.

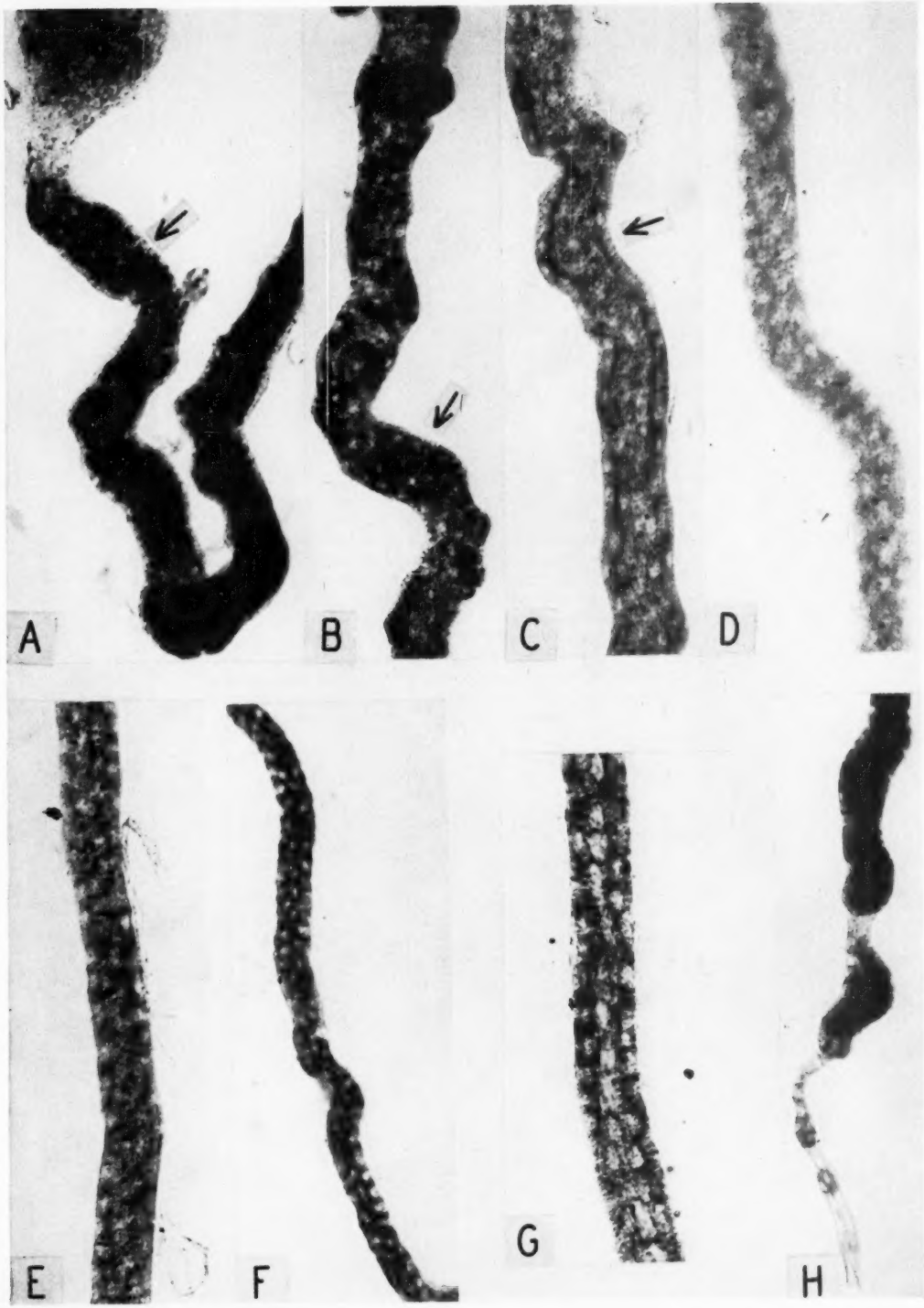

A
B
C
D
E
F
G
H

PLATE 3. CRUSH ANURIA—MAN BURIED UNDER DEBRIS FOR SEVERAL HOURS

Fracture of spine with compression of cord; passed a few oz. of urine each 24 hours and died with a blood urea of 420 mg. per cent (*cf.* J. Path. & Bact., 1941, **53**, 493, Case 1). Specimen through the courtesy of Dr. J. E. Morison, Belfast, N. I.

A complete proximal convolution with glomerulus. The patchiness of the staining of the mitochondrial pattern is evidence of the damage to the tubular epithelium. At the arrows this damage is so intense as to have resulted in disruption of the basement membrane and the epithelium is reduced to granular debris. In spite of these areas of tubular destruction, note intervening stretches with normal nuclei and mitochondrial apparatus (A). Areas of damaged and well preserved tubule alternate irregularly to the terminal medullary portion of the convolution (B). Here the basement membrane is barely visible and there is complete destruction of the renal epithelium; the irregular black stained spots are clumps of cellular debris and detritus. Magnification 80 ×.

The insert shows the detail of an incipient disruptive lesion. Magnification 200 ×.

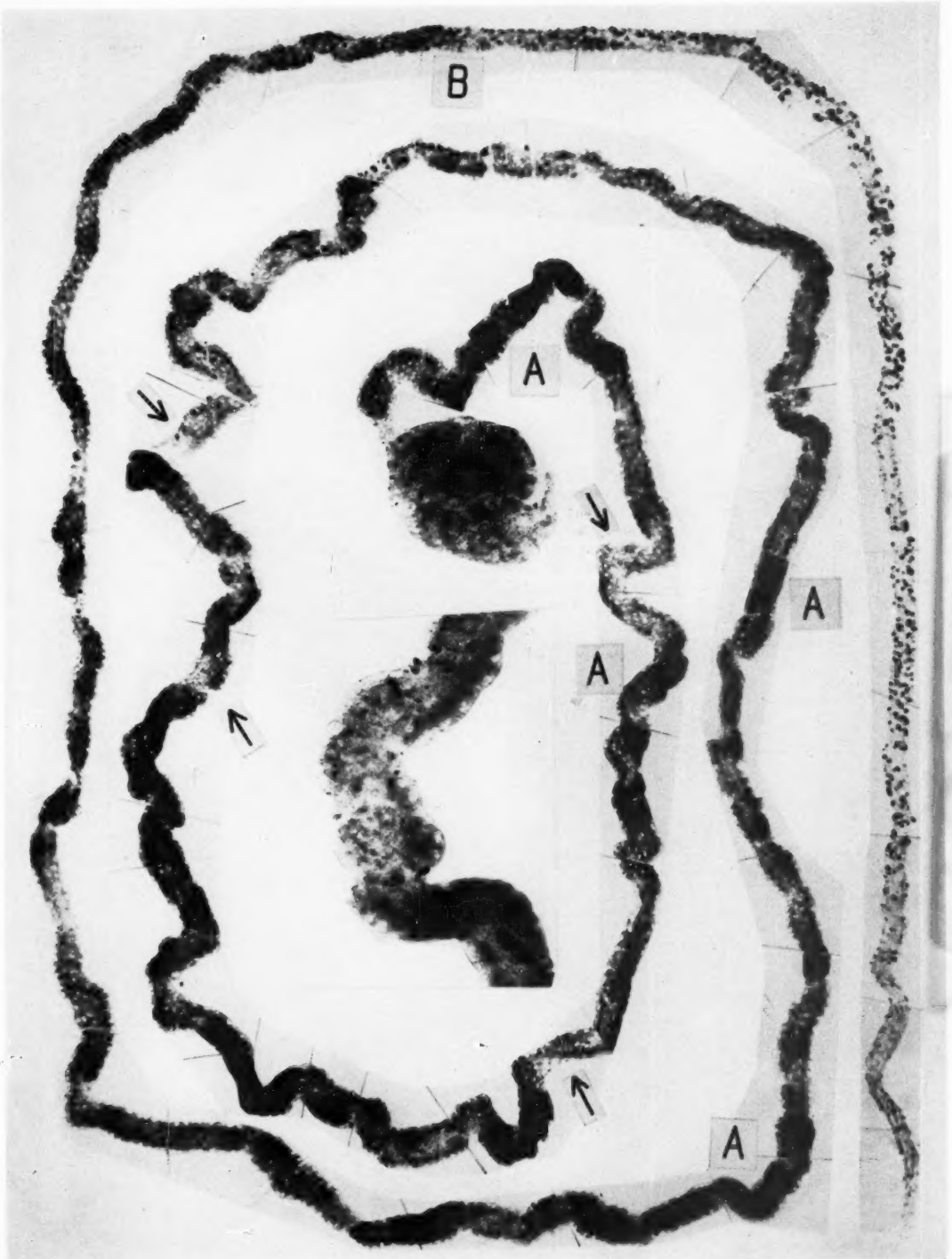

B
A
A
A
A
A

PLATE 4. A SPRAY OF DISTAL CONVOLUTIONS JOINING TO FORM A COLLECTING TUBULE FROM THE SAME KIDNEY AS IN PLATE 3

Four ascending limbs with normal nuclear pattern and tubular contour rise on the left to pass into the distal convolutions. Note the rupture of the basement membrane and tubular disruption in all four at the arrows; each distal is distended with a heme pigment cast (A) of varying length; note lack of cast or pigment at points of tubular damage and the lack of disruption where the casts distend the tubule. The cast is continuous in the upper two distals far into the collecting tubule. Magnification 40 ×.

Insert shows detail of disruptive lesion in lower distal convolution. Magnification 100 ×.

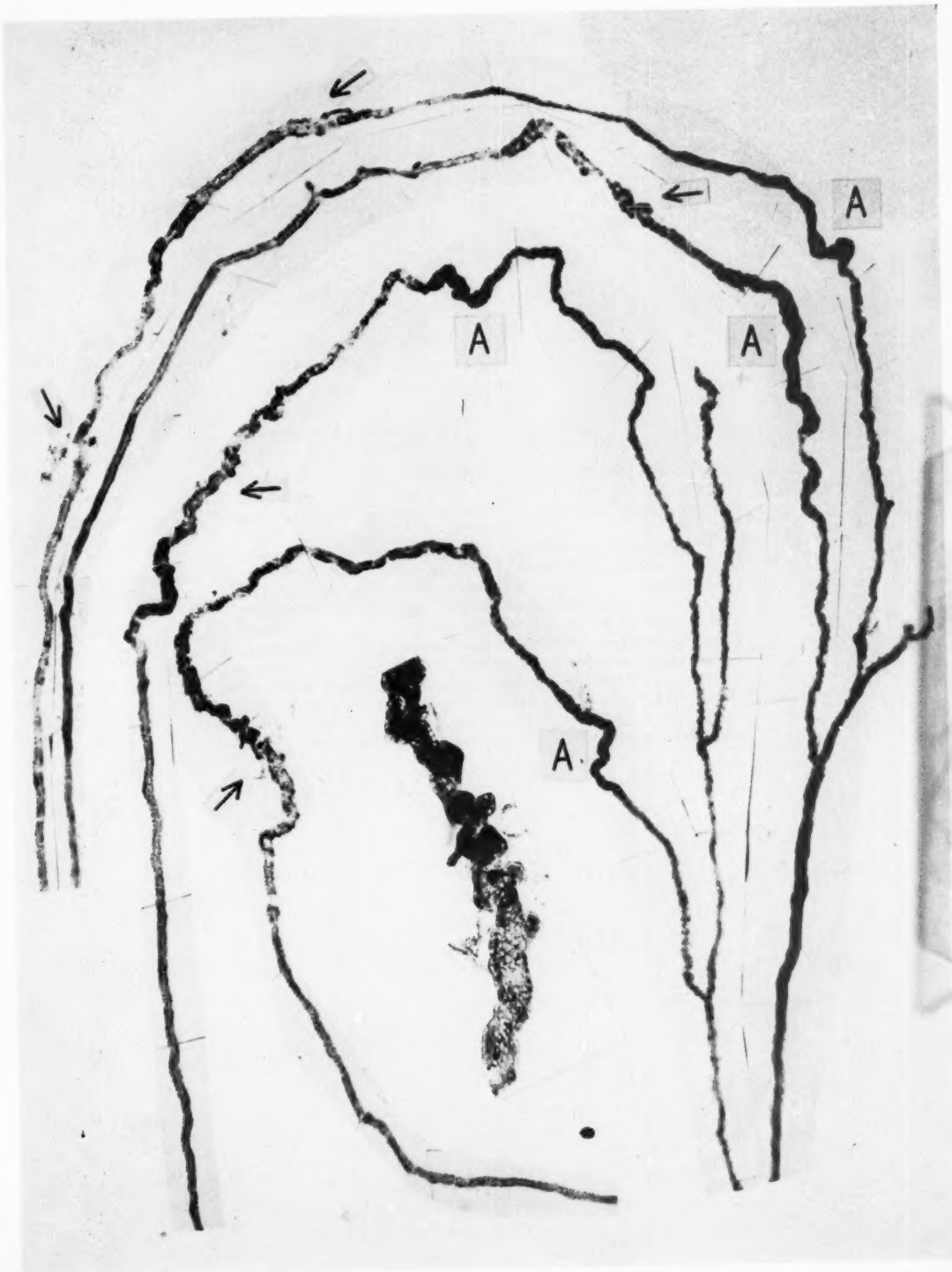

A
A
A
A

PLATE 5. CRUSH ANURIA—YOUNG WOMAN PINNED UNDER A BEAM FOR SIX HOURS

Legs crushed; shock. Lived nine days, urinary output 120 to 420 cc. per 24 hours, terminal blood urea 368. (*Cf.* Lancet, 1941, **2**, 549, Case 1). Specimen through the courtesy of the late John Shaw Dunn of Glasgow, Scotland.

The appearance of the disruptive lesion in the distal convolution from the first case in which it was described by Dunn, Gillespie and Niven (8). An ascending limb with a fairly well preserved nuclear pattern curves down and then up to the distal convolution. At this point (A) the basement membrane is ruptured and the tubular wall disrupted. There is no heme cast at this point of damage but at B the tubule is distended with a black pigment cast and there is an extensive fragmentation of the tubule wall. Surrounding the fragmented tubule are granulomatous adhesions in which large capillary venous channels can be seen. Another fragmentation follows C; irregular masses of regenerating epithelial cells can be seen in the tubule below this point (D) but the tubule wall is not repaired. Magnification 81 ×.

A
B
C
D

PLATE 6. FATAL BURNS—MAN SUSTAINED MULTIPLE THIRD DEGREE BURNS OVER 1/6 OF HIS BODY

Urinary output 600 cc. per day until death on ninth day. Specimen through the courtesy of Dr. Baldwin Lucké.

A complete proximal convolution with its glomerulus. In the first quarter of the convolution the nuclear and mitochondrial pattern is essentially normal; from this point on there is extreme damage. At A the tubule is swollen, the basement membrane broken, the renal epithelium necrotic, and the tubular wall disrupted. Varying degrees of tubular damage alternate until the terminal portion of the convolution is reached (B); the basement membrane shows almost complete dissolution and only irregular clumps of nuclear and cellular debris remain. Magnification 83 ×.

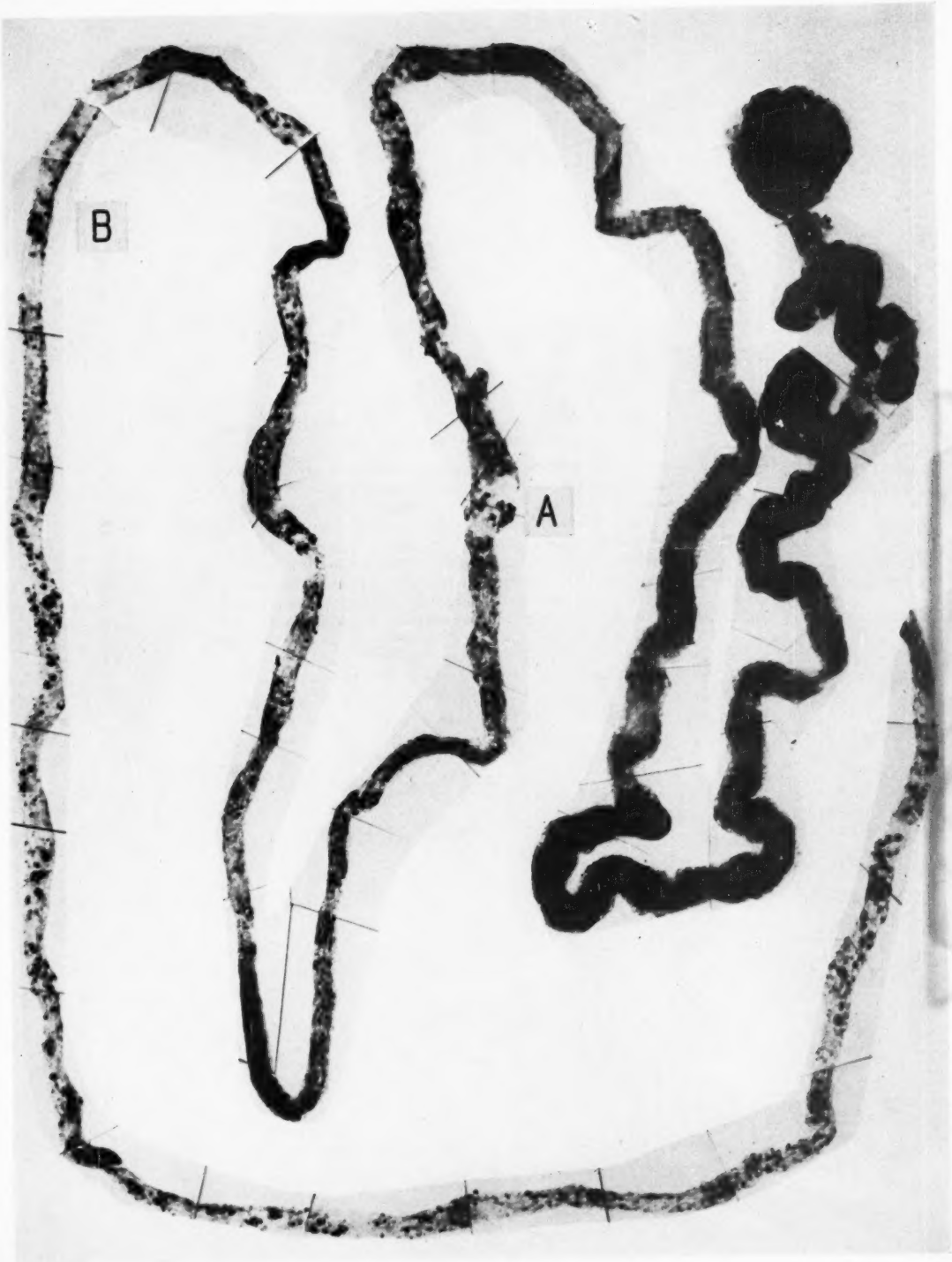

B
A

PLATE 7. THE ASCENDING LIMB AND DISTAL CONVOLUTION OF THE SAME NEPHRON AS IN PLATE 6

The former curves from right to left: it is fairly well preserved in its first portion but from A its basement membrane is fragmentary and the epithelium necrotic and desquamated. In the distal convolution there is a segment of tubular disruption with fragmentation of the basement membrane (B); note that there is no associated heme cast or deposit in the tubule lumen. Where these objects occur (C) there is no tubular disruption.

The damaged terminal segment of the proximal convolution shown in the preceding figure and the ascending limb of this figure lay side by side in the subcortical medulla. It is commonly stated that the distinction between them in the histological section is not difficult, an opinion which may be judged by comparing the two figures. Magnification 83 ×.

Insert shows detail of disruptive lesion at a magnification of 200 ×.

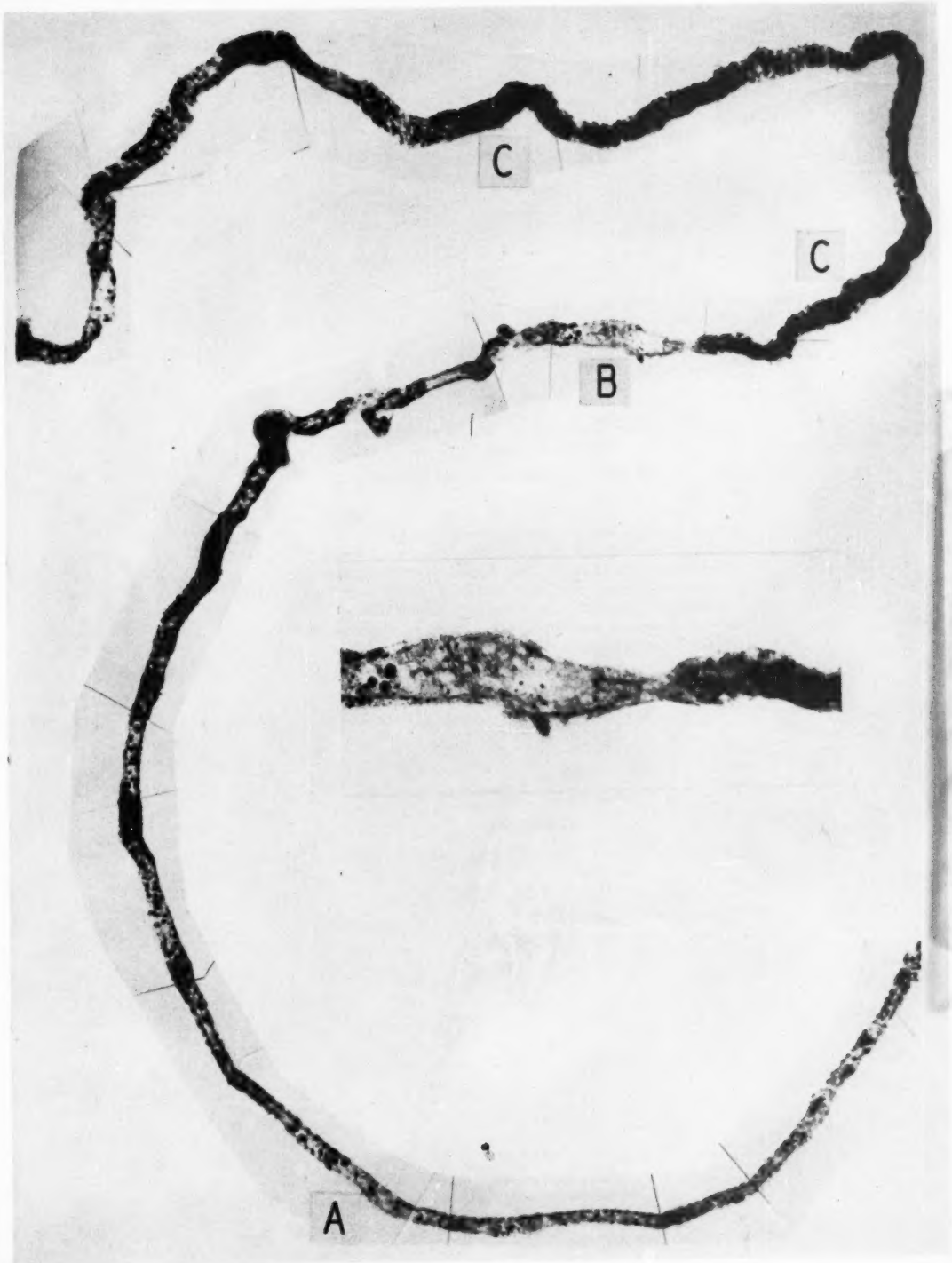

C
C
B
A

PLATE 8. FATAL BURNS—MAN BURNED BY GASOLINE EXPLOSION, FACE, NECK, SHOULDERS AND BACK TO WAIST

Died in renal failure on ninth day. Specimen through the courtesy of Dr. N. Winer.

The insert shows a *Camera lucida* tracing of a complete nephron. The cross-hatched portion of the tubule indicates the seat of extreme tubular disruption as illustrated in the photographs. As evidence of the random distribution of the disruptive lesions note that the terminal segment of the proximal convolution was intact in this specimen. The distal convolution contained no heme casts and showed no apparent lesions. In Inserts 1, 6–8 the nuclear patterns are normal, but obscured by the dense black staining of the mitochondrial material. Magnification 83 ×.

Winer Burn Kidney
126391
3
2
1
4
5
6
7
8

PLATE 9. UTERINE HEMORRHAGE—COPIOUS HEMORRHAGE IN THIRD STAGE OF LABOR; SHOCK

Plasma, whole blood, and haemoglobin solution given intravenously. Urine volume on fourth day 120 cc.; 360 cc. on the sixth. Died on the ninth day. Specimen through the courtesy of Dr. Baldwin Lucké.

Glomerulus and proximal convolution. The greater part of the proximal convolution is well preserved: the heavy staining was deliberately done to bring out the contrast of two regions (A) where stretches of the convolution show damage. In the more proximal a slight disruptive lesion is shown; in the second there is complete disruption of the tubule wall with surrounding tissue reaction. Magnification 83 ×.

Insert shows incipient disruptive lesion. Magnification 200 ×.

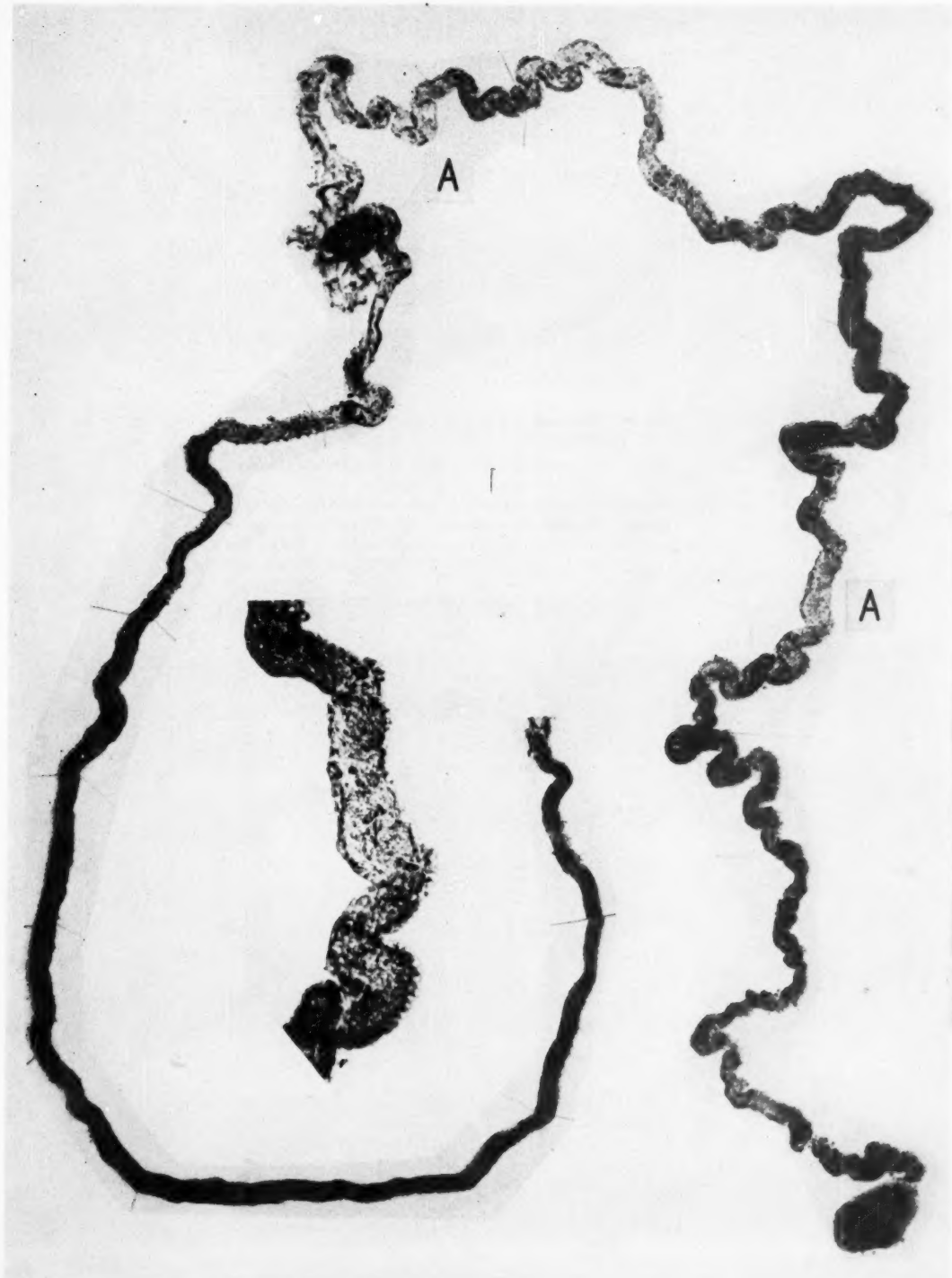

A
A

PLATE 10. ASCENDING LIMB AND DISTAL CONVOLUTION FROM THE SAME KIDNEY AS IN PLATE 9

The first portion of the ascending limb is intact, from A it shows extensive damage. At B is complete disruption of the distal convolution with surrounding adhesions of reactive granulation tissue in which are large capillary-like channels. The dense black segments of the greater part of the convolution are tubule filled with heme cast; as is so commonly the case there is no apparent lesion in the tubule in the proximity of the pigment casts. Magnification 84 ×.

Insert shows detail of disruptive lesion at a magnification of 200 ×.

B
A

PLATE 11. TRANSFUSION REACTION—TRANSURETHRAL PROSTATECTOMY, SHOCK

B.P. 78/60. Three blood transfusions. Urine output from retained catheter not measured. Died on fifth day following operation with "marked elevation of blood chemistry."

A complete proximal convolution. The glomerulus is missing. The most striking feature shown by this specimen is the random scattering of stretches of disrupted tubule (A) among portions in which the nuclear pattern indicates a relative normality of the tubule wall. (B) The very dark stretch of tubule is due to the presence of haemoglobin in the renal epithelium. Magnification 82 ×.

B
A
A
A
A
B
B

PLATE 12. THE TERMINAL SEGMENT OF ANOTHER PROXIMAL CONVOLUTION FROM THE SAME KIDNEY AS IN PLATE 11

Regeneration has replaced the original epithelium by cells with oval nuclei and with sparce mitochondrial material. The new epithelium had absorbed haemoglobin as is evident from the dark granules which were positive for free iron with the Berlin Blue reaction. At A debris impregnated with blood pigment, negative for free iron but positive to benzidine, fills and distends the tubule lumen. Note that as is usual, these substances have caused no damage to the epithelium of the tubule wall. Magnification 82 ×.

Insert shows detail of pigment in regenerated cells at a magnification of 200 ×.

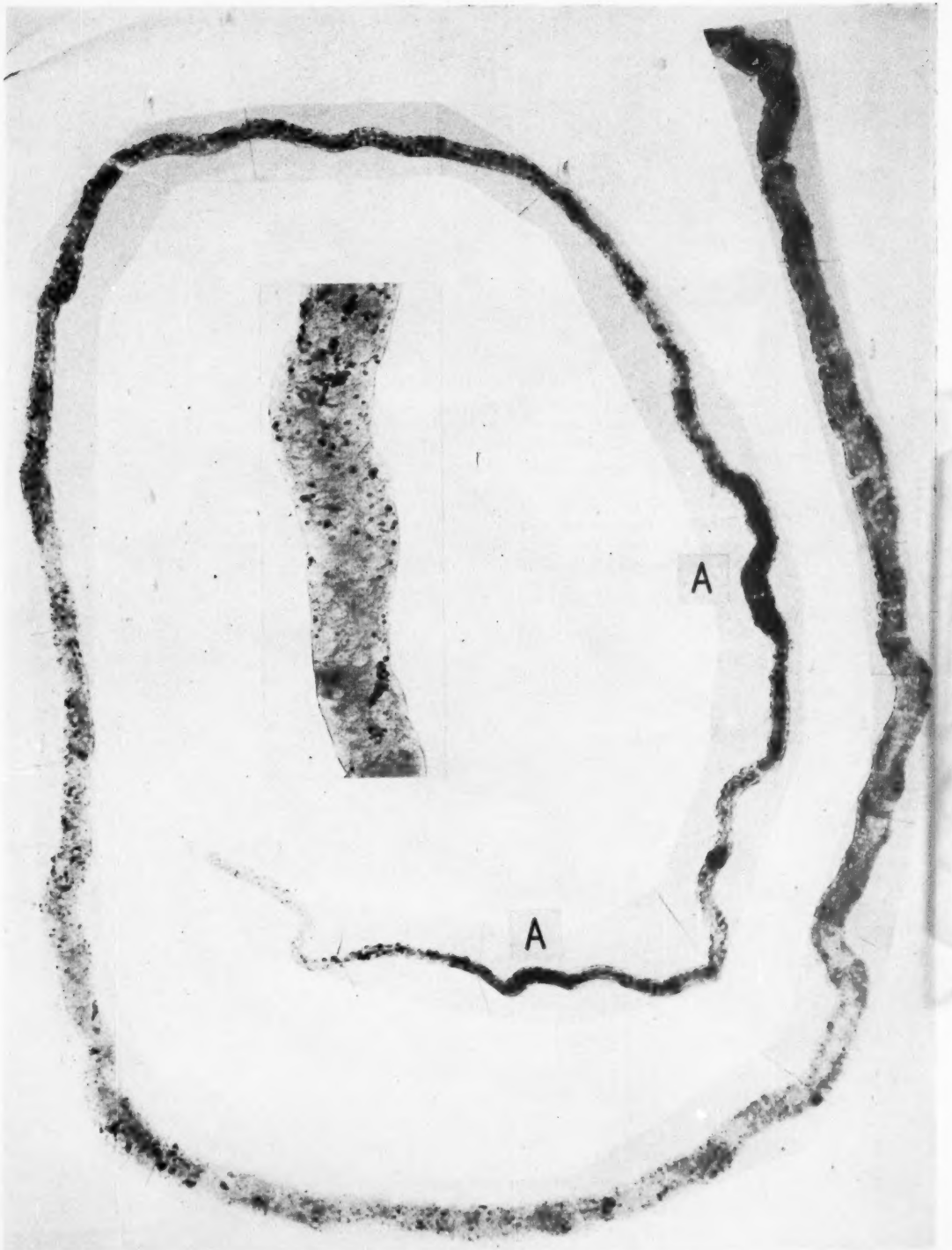

A
A

PLATE 13. BLACKWATER FEVER—MAN BROUGHT FROM THE COUNTRYSIDE WITH A TYPICAL HISTORY OF BLACKWATER FEVER; DIED SHORTLY AFTER ADMISSION TO THE HOSPITAL

Specimen through the courtesy of Col. H. C. Clarke, Canal Zone, Panama.

This and Plate 14 show a complete proximal convolution with its glomerulus. At the very origin of the convolution dilatation of Bowman space, dissolution of the basement membrane and destruction of the tubule wall are seen (A). There then follow irregularly alternating disruptive lesions and stretches of convolution which are relatively better preserved. Magnification 82 ×.

A

PLATE 14. THE DISTAL HALF OF THE CONVOLUTION (SEE PLATE 13)

This shows more damage and the terminal medullary portion is the seat of an almost continuous disintegration of the basement membrane and necrosis of the tubular epithelium. In areas of relatively normal tubule, droplets of absorbed heme pigment are visible, some positive to the Berlin Blue reaction and some to benzidine. These stain black with iron haemotoxylin and therefore are obscured in the upper portions of the convolution by the mitochondrial elements; where the latter are less dense (A), the fine pattern of heme pigmentation can be seen. The dark granular material in the desquamated dead cells in the lumen of the tubule (B) was positive to the Berlin Blue reaction. Magnification 82 ×.

Insert shows detail of heme pigment granules at A at a magnification of 200 ×.

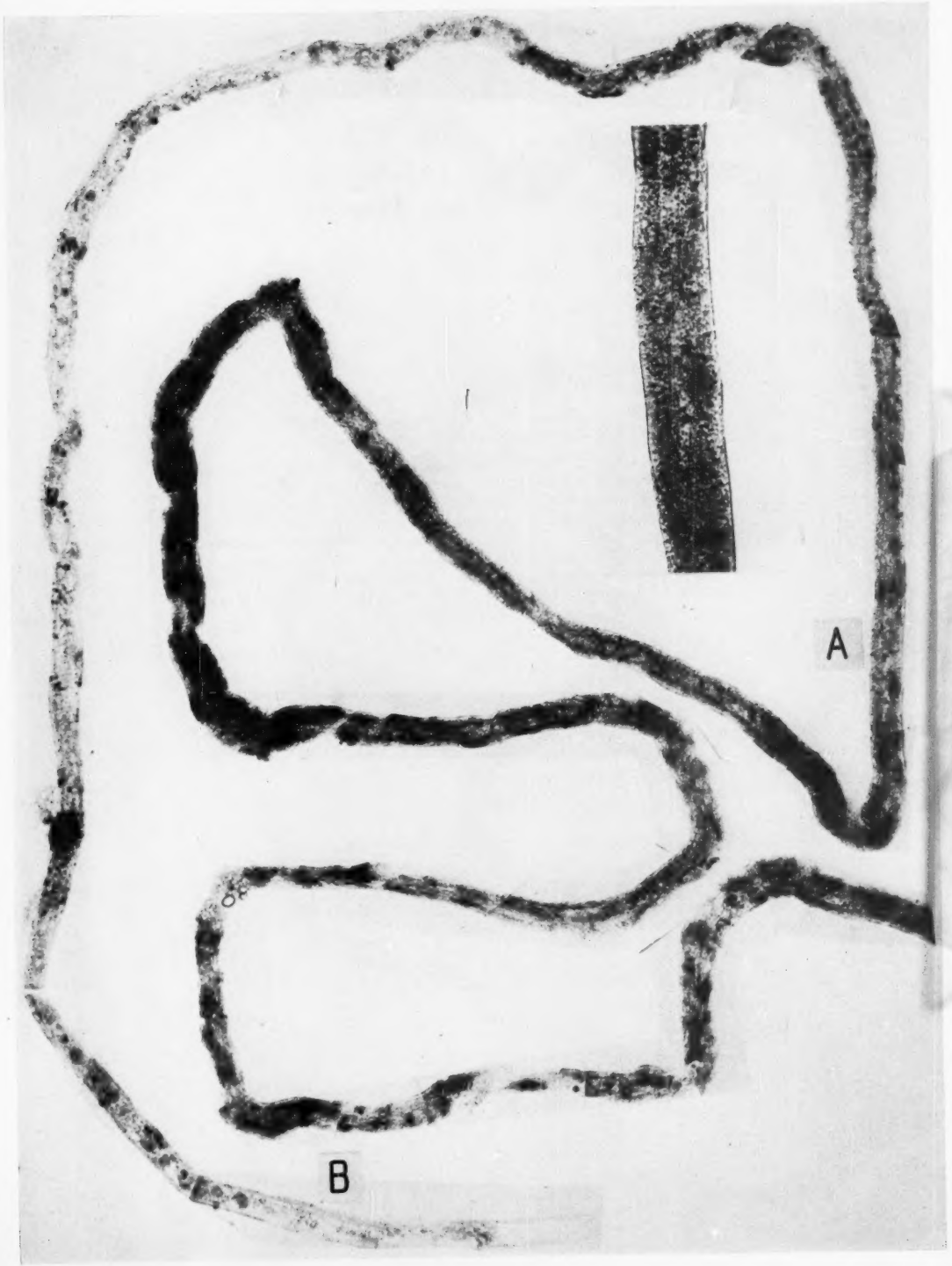

A
B

PLATE 15. ASCENDING LIMB AND DISTAL CONVOLUTION FROM THE SAME KIDNEY AS IN PLATES 13 AND 14

A dilated ascending limb on the left, lined by an atypical epithelium. Note the similarity of this abnormal distal portion of the nephron to the abnormal regenerated terminal portion of the proximal convolution in Plate 12, except that the ascending limb does not contain granules or droplets of absorbed heme pigments. A complete dissolution of basement membrane, necrosis of epithelium and tubular disruption in the distal convolution at A. Scattered along the length of the entire specimen are collections of heme pigment impregnated debris. (B) As is usual, the tubule wall is less damaged at these points. Magnification 82 ×.

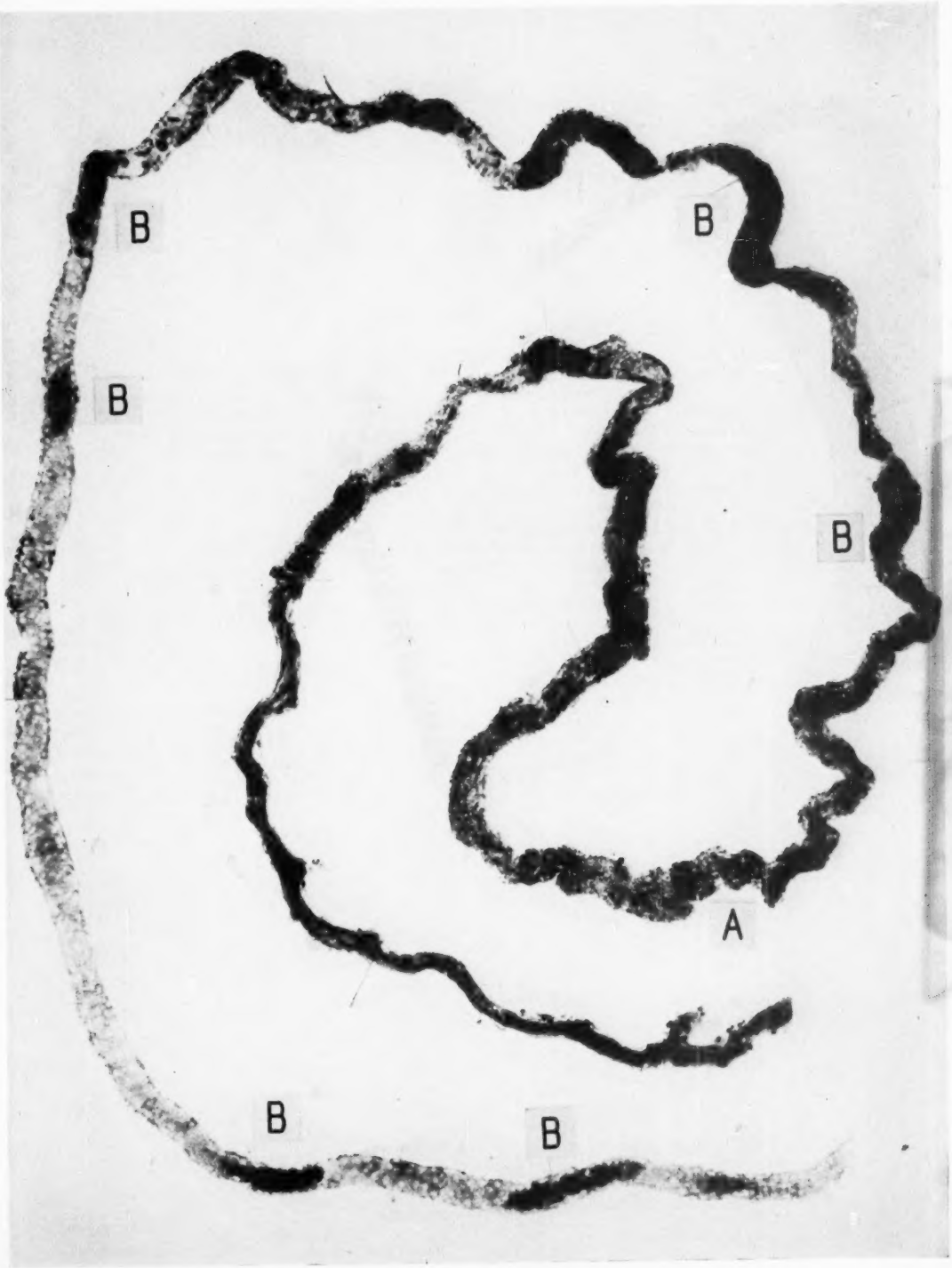

B
B
B
B
A
B
B

PLATE 16. SUBLIMATE POISONING

Details of clinical history in text, page 1314. Died on the 29th day.

The terminal medullary portion of a proximal convolution. The cortical portion of this convolution showed the typical nephrotoxic necrosis due to mercury. This lesion is visible in the horizontal stretch of tubule above, A, an intact basement membrane outlining the dead mass of dark stained necrotic debris. At B there begins a long segment of tubule, greatly swollen, in which the basement membrane has completely disintegrated and the tubule wall is destroyed. The tubular disruption ends abruptly (C) and the convolution continues, its basement membrane intact, to the passage into the thin loop of Henle's loop. At the point of greatest disruption (B^1) irregular regeneration of the epithelium is evident in the clusters and masses of cells with large vesicular nuclei. It is also evident that this regeneration has not repaired the tubule wall or reestablished a lumen; this, apparently due to lack of a basement membrane to direct the course of the proliferating cells. Magnification 82 ×.

Insert B^1 shows the disruptive lesion at B^1 at a magnification of 200 ×. For the appearance in histological section of a similar lesion *cf.* Figure 1.

Insert A shows a typical nephrotoxic tubular lesion in the terminal segment of a proximal convolution of a nephron from the kidney of a dog which had received an intravenous injection of 75 mg. of corrosive sublimate. The renal epithelium is completely necrotic and reduced to granular debris. Note the intact basement membrane, at places out of focus and therefore invisible, which maintains the tubular contours. Magnification 200 ×.

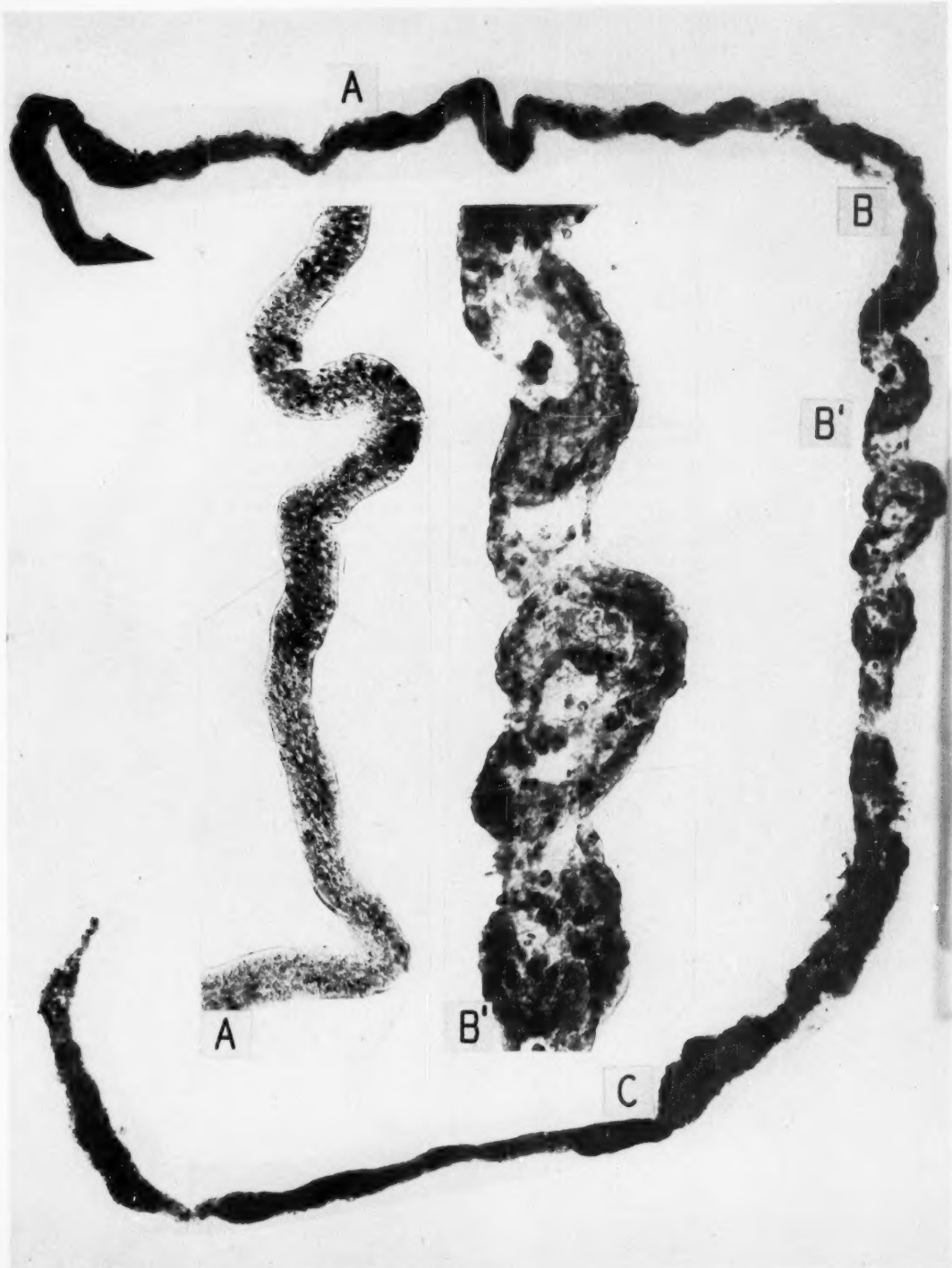

A
B
B'
A
B'
C

PLATE 17

TWO DISTAL CONVOLUTIONS AND THE ORIGIN OF THE COLLECTING TUBULE FROM THE SAME KIDNEY AS IN PLATE 16

At the lower right a distal convolution is filled in great part with dark staining casts which in histological section stained with eosine and contained no heme pigment. The second half of the upper distal convolution is similarly filled and the cast extends some distance into the collecting tubule on the extreme left. The original thick epithelium of the first portion of the upper distal convolution has been replaced by a flat atypical regenerated layer of cells which has not completely healed the disruption that had occurred in the tubule wall (A). Magnification 83 ×.

A
A

PLATE 18. POTASSIUM CHLORATE POISONING

Details of clinical history in text, page 1316. Died on the 11th day.

A complete proximal convolution. The dark staining poorly preserved mitochondria in the first half of the convolution indicates a relative integrity of the tubular epithelium, although there may be some lesser form of damage such as "cloudy swelling." Beginning at the arrow there is a frank necrosis of the tubular epithelium with desquamation which continues to the termination of the convolution in the narrow limb of Henle's loop. Magnification 84 ×.

For the appearance in histological cross section of the partially necrotic upper half of a similar convolution, *cf.* Figure 2.

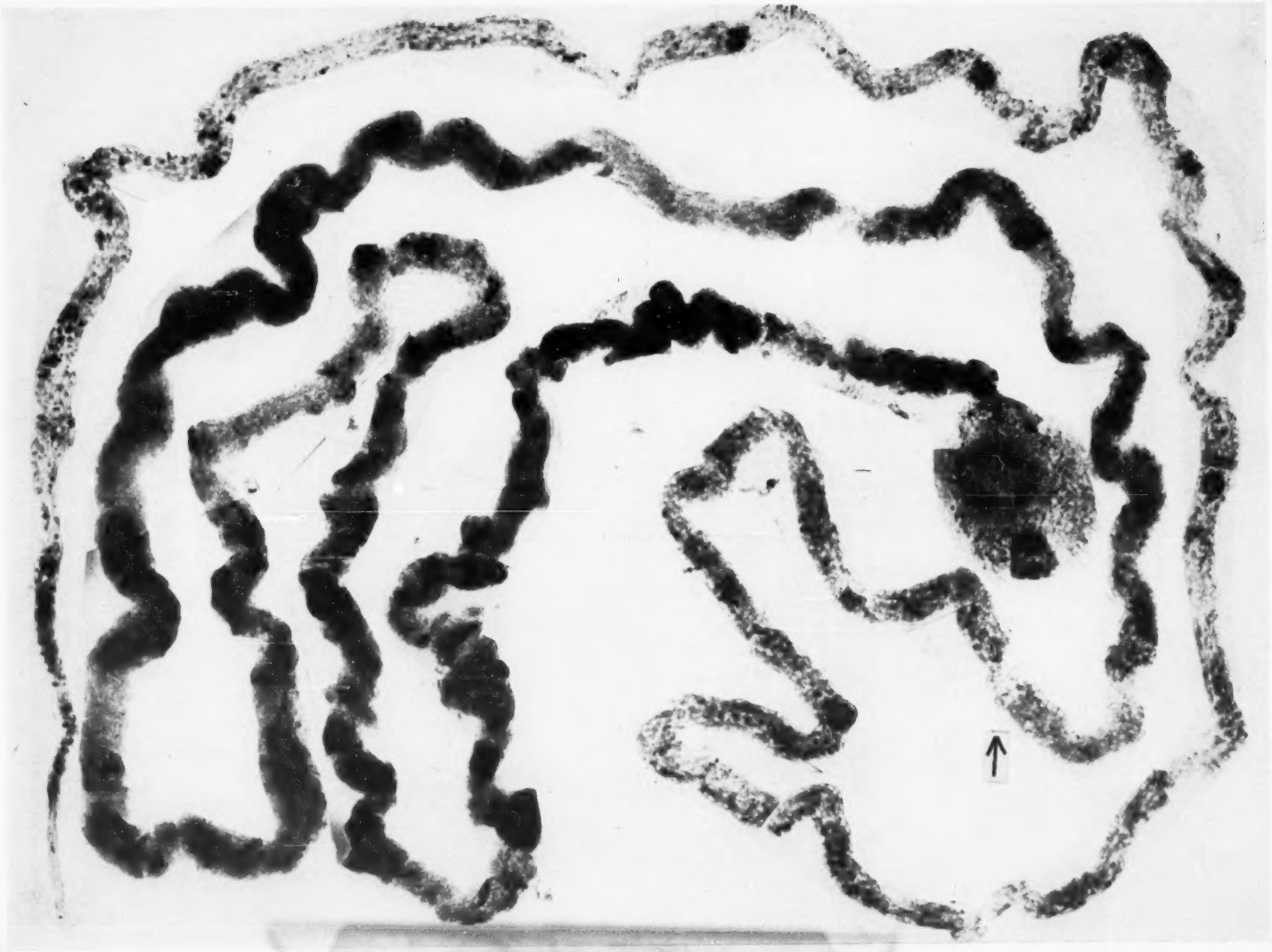


PLATE 19. AN ASCENDING LIMB, BELOW, AND DISTAL CONVOLUTION FROM THE SAME CASE AS IN PLATE 18

The ascending limb is dilated but its cellular pattern is fairly well preserved. At A is a huge cast which blocks the distal convolution and proximal to it a greatly dilated segment with complete disintegration of the basement membrane and disruption of the tubule wall. Note the abortive regeneration of sheets of cells that grow at random. Magnification 84 ×.

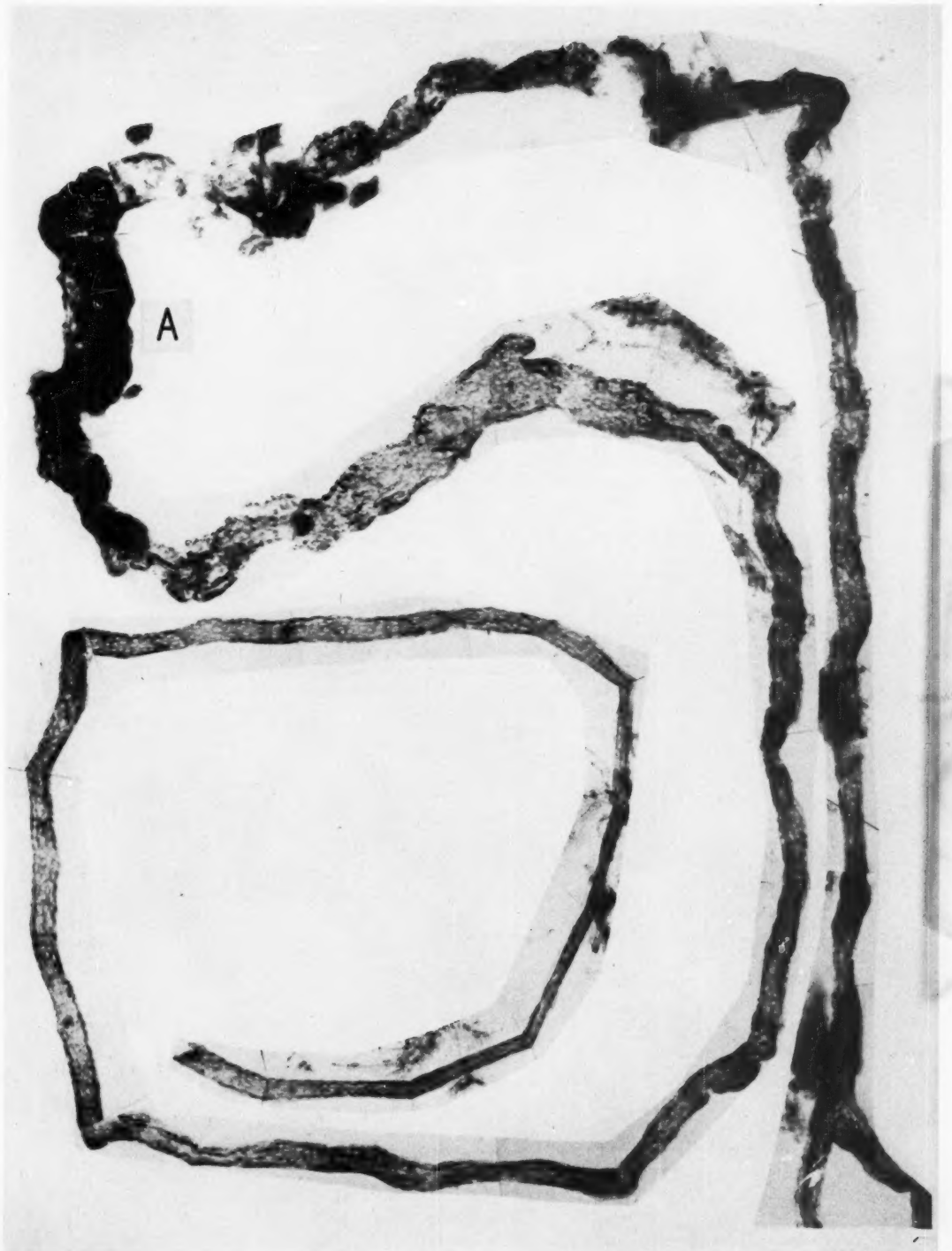

A

PLATE 20. SULFONAMIDE INTOXICATION

Woman with sinusitis and a previous history of albuminuria took 3.4 gm. sulfathiazole in one day at home, and 3 gm. after entering hospital for severe headache and pain in back. Did not void in 12 hours, erythematous rash appeared, 60 cc. urine by catheter. Died 20 hours after admission (*cf.* J.A.M.A., 1942, **119**, 8, Case 4). Specimen through the courtesy of Dr. Max Lederer.

A complete proximal convolution and its glomerulus. As was noted by Dr. Lederer in histological section there is necrosis of the renal epithelium throughout almost the complete length of the convolution. The basement membrane, however, is intact and its tube is stuffed with cellular debris in various degrees of consolidation. This case showed in extreme degree damage of the proximal convolution: as noted in our text there is great variation in the type of reaction of the kidney to the deleterious effects of the sulfonamides and many do not show the nephrotoxic effect on the proximal convolution that is well illustrated in this case. Magnification 80 ×.

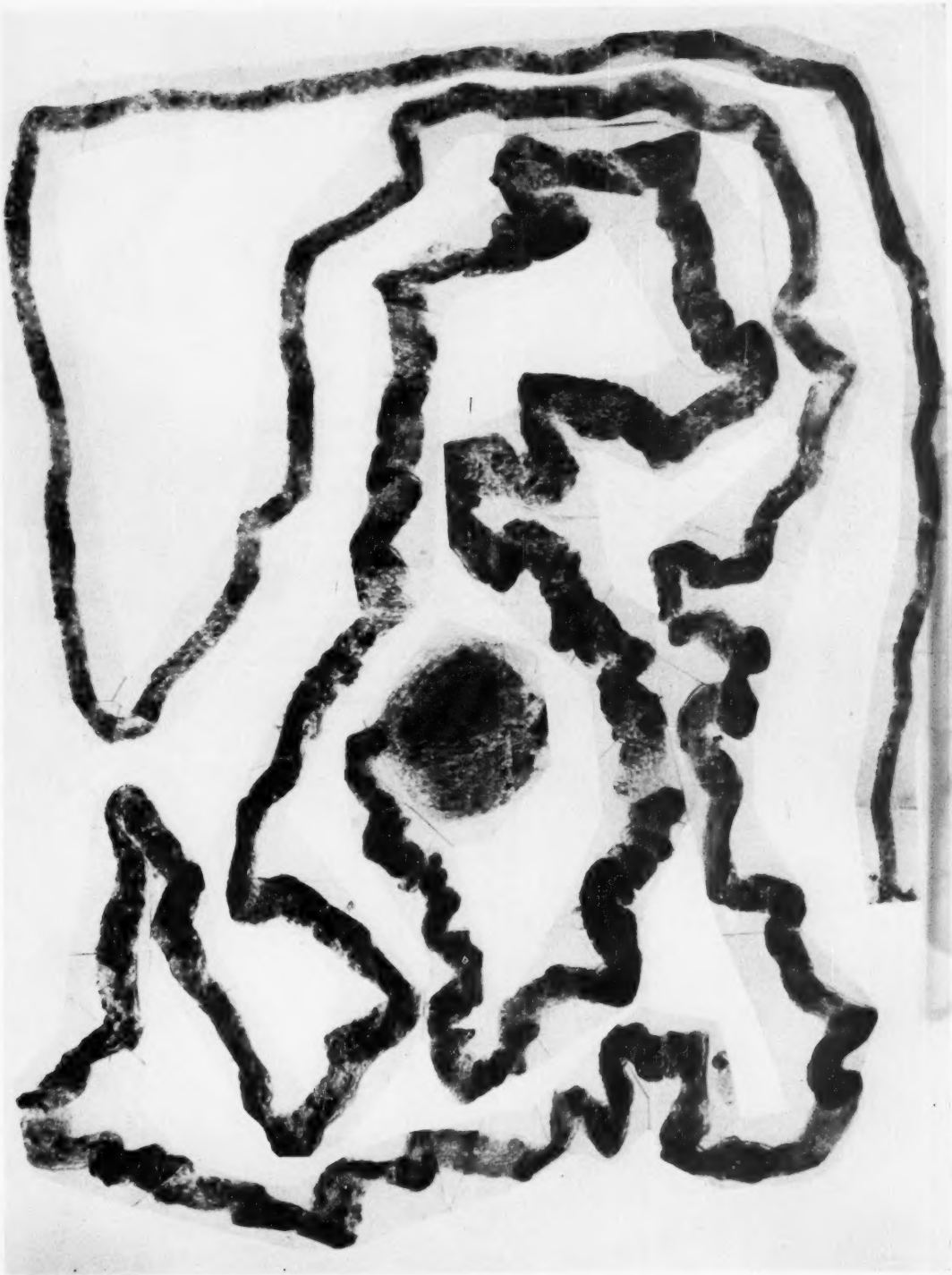


PLATE 21. SULFONAMIDE DAMAGE—MALE ADMITTED WITH A "COLD"

Symptomless treatment with sulfathiazole one month previously. Given 13 gm. sulfathiazole in 48 hours; nausea, vomiting, urinary suppression, 150 cc. in four days, and death in uremia eight days later. Specimen through the courtesy of Dr. Baldwin Lucké.

Ascending limb and two distal convolutions and collecting tubule. The ascending limb (left) shows an intact epithelium. At A and A^1 the distal convolution shows the disruptive lesion; at B there is a cast distending the intact tubule. At A a projecting sheet of atypical epithelial cells can be seen but the disruption is not filled. The other distal convolution, above and to the right, shows less damage; at C there is an incipient solution of continuity of the basement membrane. In the collecting tubule, below, the typical epithelial pattern is well preserved; the lumen contains masses of debris and desquamated epithelial cells. Magnification 83 ×.

Insert shows detail of disruptive lesion at A at a magnification of 200 ×.

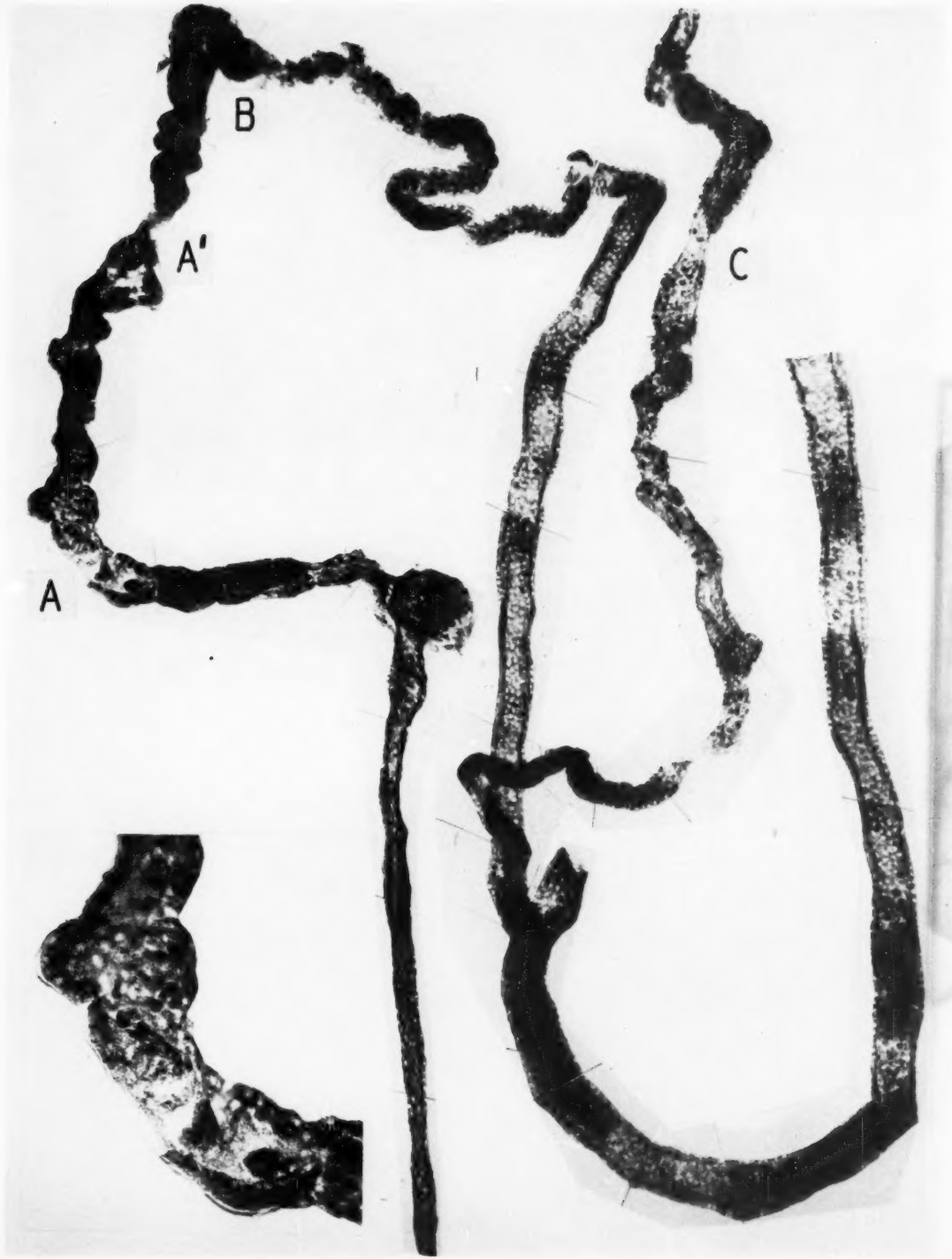

B
A'
C
A

PLATE 22. DI-ETHYLENE GLYCOL POISONING

Details of history in text, page 1322. Died on the 12th day. Specimen through the courtesy of Dr. Milton Helpern.

A portion of the proximal convolution. The dissection of proximal convolutions from this kidney was exceedingly difficult because there was a combination of a nephrotoxic vacuolar necrosis of the epithelium and a disruption of the basement membrane (A) throughout the convolution. For the appearance in histological section of the lesion see Figure 3. The specimen was under-differentiated for purposes of contrast as the almost complete vacuolization of the renal epithelium left little to stain by the usual procedure. For the typical appearance of a lesser and limited lesion of di-ethylene glycol under experimental conditions, see Plate 44. Magnification 84 ×.

A
A
A

PLATE 23. ASCENDING LIMB WITH DISTAL CONVOLUTION ON THE RIGHT JOINING ANOTHER TO FORM A COLLECTING TUBULE; FROM THE SAME CASE AS IN PLATE 22

There are casts of debris in the ascending limb and in other portions of the various tubules at A. At B the distal convolution is greatly swollen, the basement membrane disintegrated and the tubule disrupted. The distal convolution on the left is fairly well preserved; the wall of the collecting tubule is intact and its lumen contains debris and a consolidated cast. Magnification 80 ×.

Insert shows detail of disruptive lesion at B at a magnification of 200 ×.

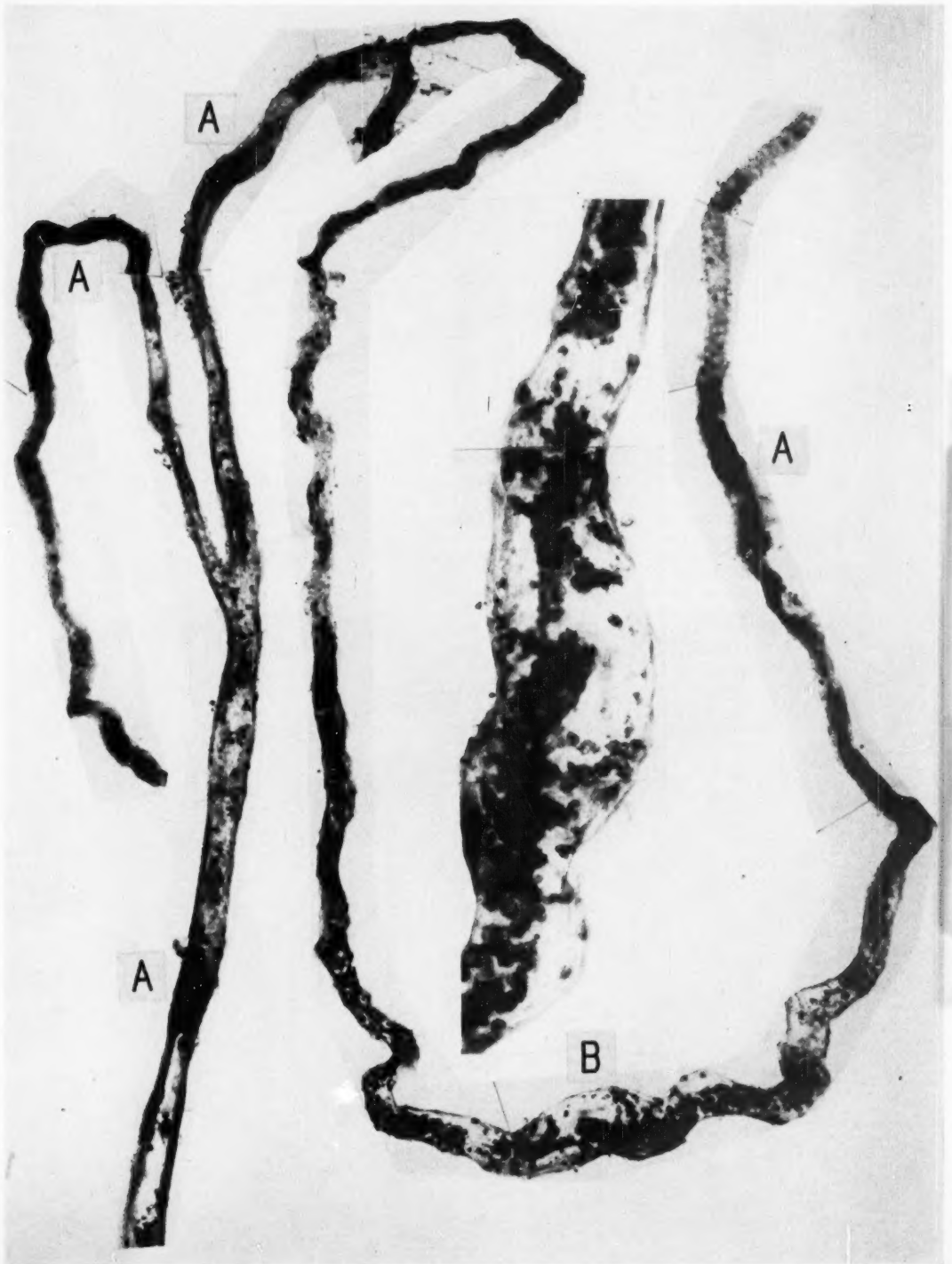

A
A
A
A
B

PLATE 24. DI-ETHYLENE GLYCOL POISONING—KIDNEY OF THE OTHER INDIVIDUAL WHO WAS POISONED AT THE SAME TIME BUT LIVED 24 DAYS

(See text, page 1322, for detailed history.)

Glomerulus and first half of proximal convolution. In contrast with the intensely damaged convolution shown in Plate 22, extensive regeneration of the tubular epithelium has occurred in the 12 days following the biopsy. See Figures 4 and 5 for the change that occurred in this time. The dissected specimen shows, however, the inadequacy of this marked epithelial proliferation so far as a restitution of a functioning nephron is concerned. Masses and sheets of proliferating renal epithelial cells impinge upon and occlude the lumen with the production of valve-like flaps and whorls of cells (C). As a result, irregular constrictions (A) alternate with pouch-like protrusions or diverticula (B). At many places the earlier disruption of the tubular wall persists (D) though one "side" of the tubule wall seems repaired. The contrast of the real inadequacy of this repair, as seen in the dissected nephron with what appears to be good "tubular regeneration" in the histological section (Figure 5), is noteworthy. Magnification 82×.

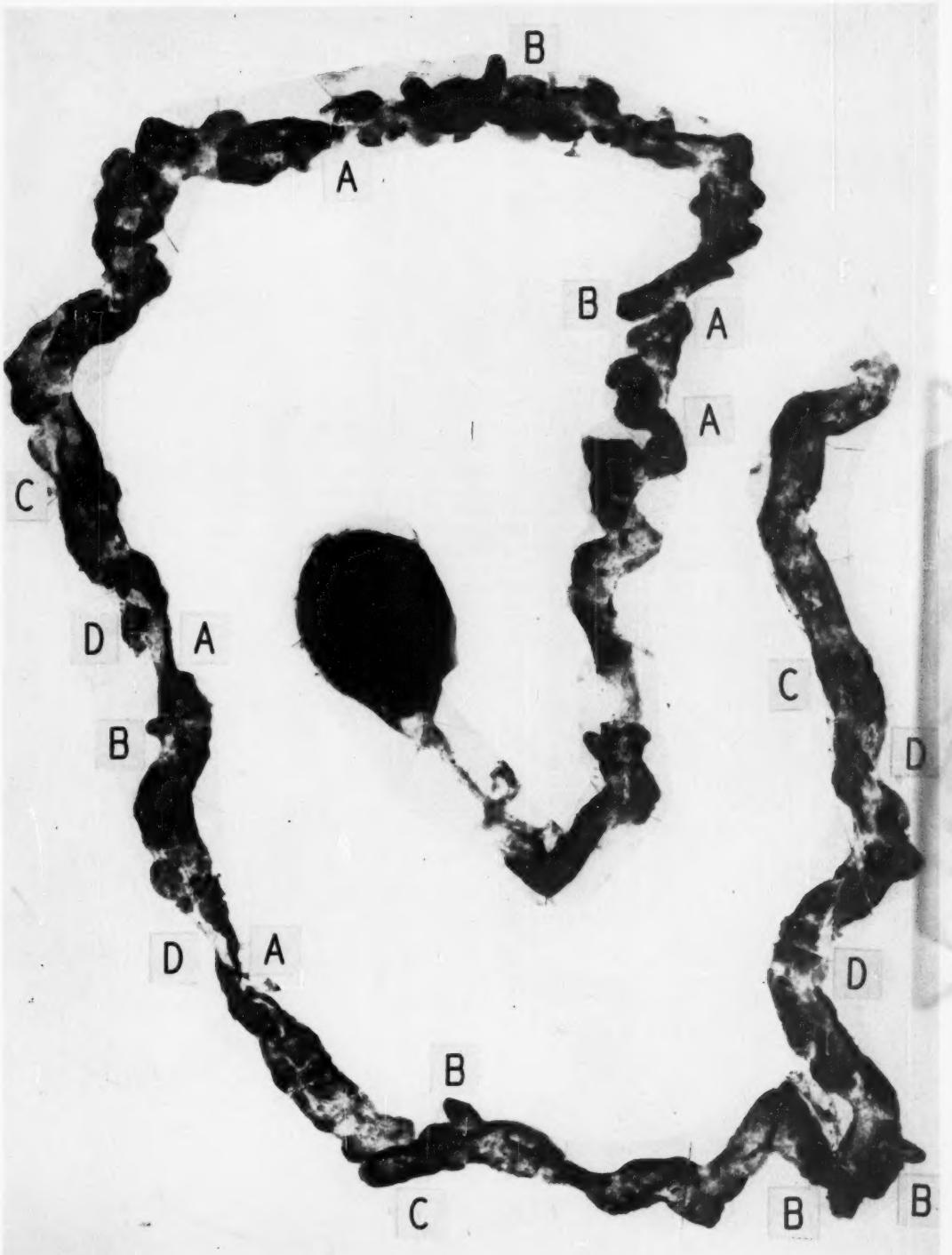

B
A
B
A
A
C
D
A
B
C
D
D
A
D
B
C
B
B

PLATE 25. ASCENDING LIMB, DISTAL CONVOLUTIONS AND COLLECTING TUBULE FROM THE SAME KIDNEY AS IN PLATE 24

Extensive casts (A) are seen in various portions of the tubules; at B and B^1 there are disruptions of the distal convolution, the latter surrounded by interstitial reaction and large capillaries. There is extensive regeneration in the ascending limb and distal convolution on the right. From B^1 to C the detail of this incomplete repair can be seen in the irregular sheets of cells that imperfectly reconstitute the tubule wall. Magnification 80 ×.

Insert shows the detail of the incomplete regeneration in the upper right distal convolution at a magnification of 200 ×.

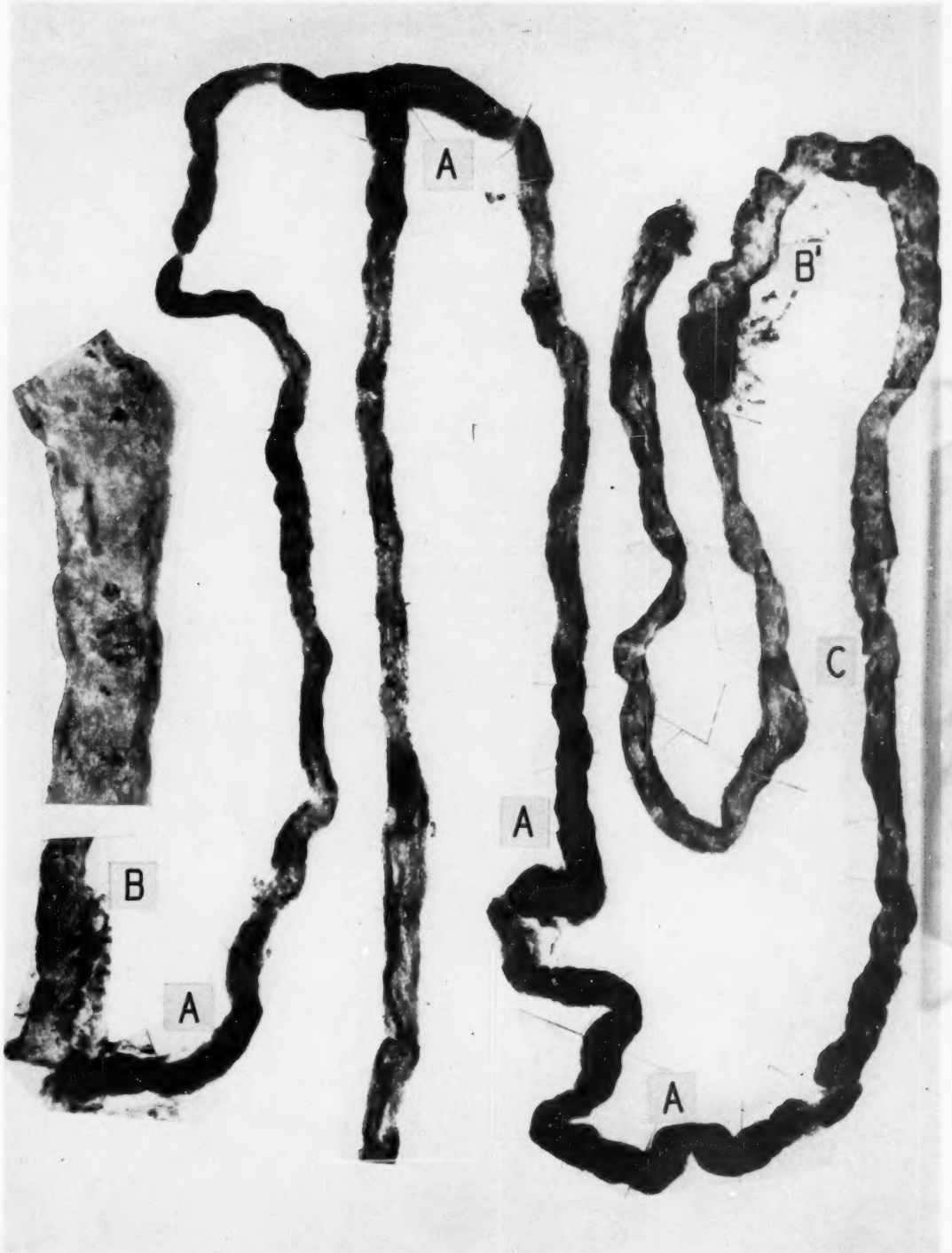

A
B'
C
A
B
A
A

PLATE 26. PAROXYSMAL HEMOGLOBINURIA

Repeated attacks of cold induced hemoglobinuria; death 12 days after a particularly severe attack. (See details in text, page 1326.) Specimen through the courtesy of Drs. Alfred Plaut and R. Sussman.

Proximal convolution and its glomerulus. This nephron came from the periphery of a fibrous scar in the kidney which, since there were no vascular lesions to account for it, was apparently the result of the damage of an earlier attack. There is an irregular atrophy and distortion of the convolution; at A there are frank disruptive lesions with peritubular adhesions. Magnification 84 ×.

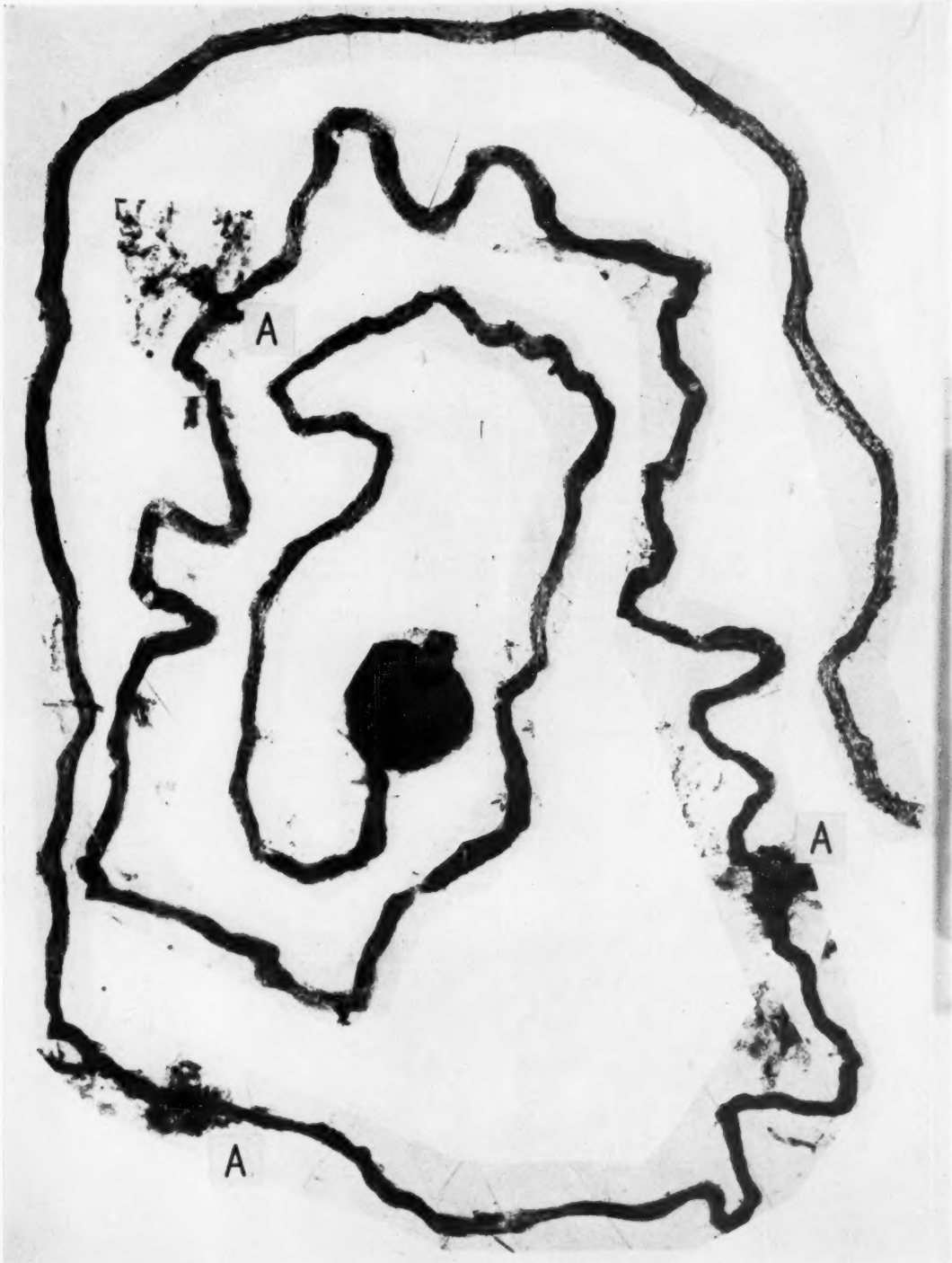

A
A
A

PLATE 27. ASCENDING LIMB AND DISTAL CONVOLUTION FROM A SIMILAR FIBROUS AREA OF THE SAME KIDNEY AS IN PLATE 26

Descending from the upper right, an ascending limb. It is difficult to decide in this atrophied and distorted nephron where the distal convolution begins, but since we have never seen diverticula elsewhere than on the distal convolution, presumably its origin is at A. There is a typical recent disruptive lesion at B with a small heme pigment cast *in situ*. These lesions were noted by Dr. Plaut in histological section and the similarity of the lesion to "crush injury nephrosis" noted. At C is a cystic dilatation of a segment of the convolution which contains some debris. Its angular irregular configuration strongly suggests the deforming effect of shrinkage of the connective tissue which surrounds it. Magnification 80 ×.

Insert shows three large diverticula on another distal convolution which have the general configuration of glomeruli. Magnification 200 ×.

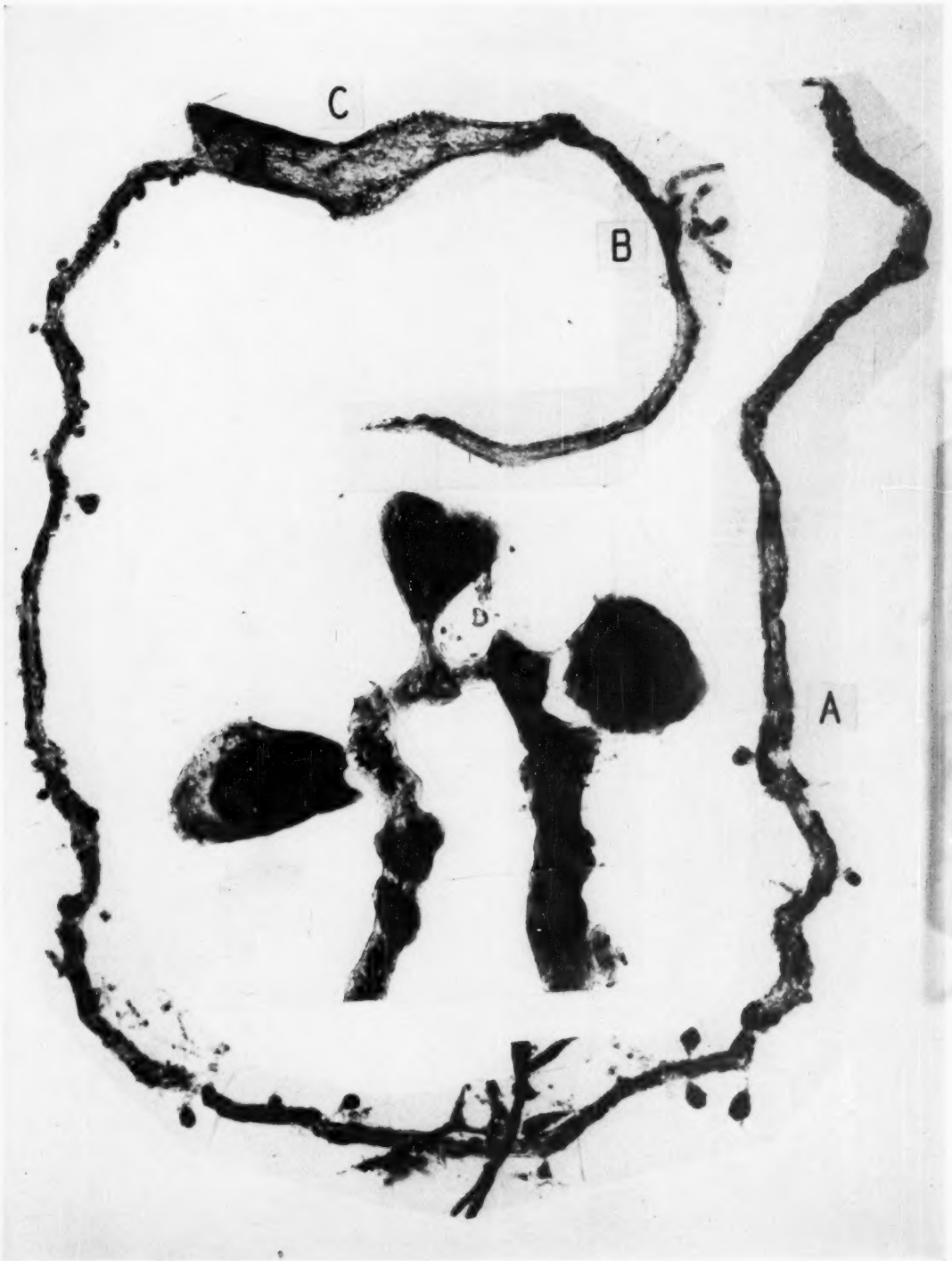

C
B
A
D

PLATE 28. DOG K29

Under nembutal anesthesia the left renal artery was clamped for 66 minutes. Seventy-two minutes after removal of clamp a 350 cc. infusion was given, consisting of 50 cc. of the animal's red cells laked in distilled water with salt added to isotonicity and 50 cc. of the animal's plasma. The animal was killed five days later.

A complete proximal convolution with its glomerulus. The first part of the convolution shows a complete disintegration of its basement membrane and disruption of the tubule wall. The convolution from here on is remarkably well preserved; note perfect delineation of the rodlets in the basal portion of the renal cells in the insert. At A this mitochondrial pattern is disturbed and at B the basement membrane is disintegrated and the cellular detail lost. This portion of the proximal convolution in the dog normally contains much visible fat and this is the source of some of the granular appearance noted in this region.

A most striking feature of the disruptive lesion due to the renal ischemia in all of the experiments, was the immediate continuity of stretches of completely damaged and perfectly preserved normal tubule; there was therefore no general toxic damage in these kidneys in spite of the infusions of haemoglobin. Magnification 82 ×.

The insert shows the perfect preservation of the mitochondrial rodlets in areas of tubule which have escaped ischemic disruptive damage. Magnification 200 ×.

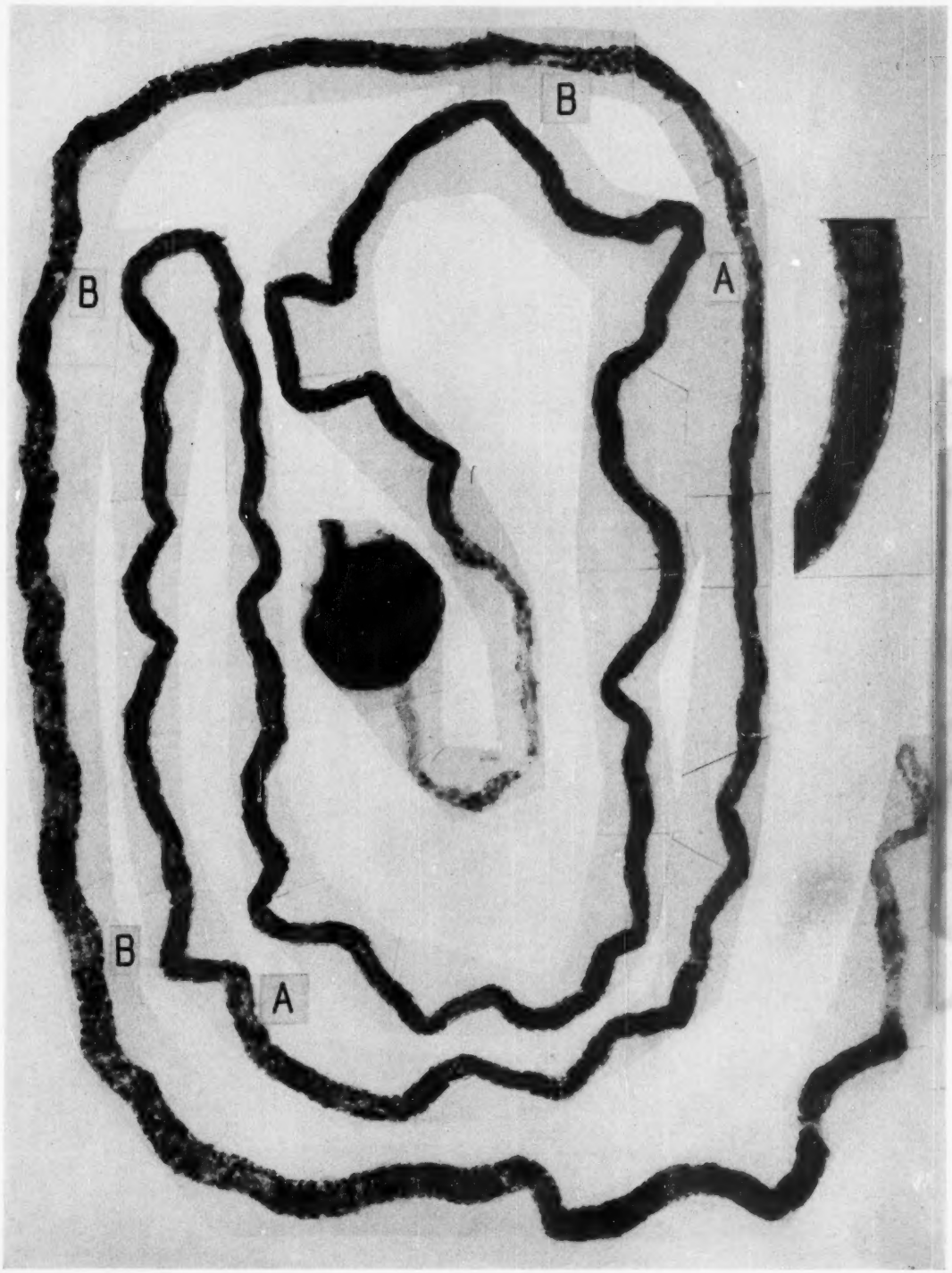

B
B
A
B
A

PLATE 29. ASCENDING LIMB AND DISTAL CONVOLUTION FROM THE SAME KIDNEY AS IN PLATE 28

The ascending limb on the left is well preserved. At A the tubule is intact but distended with heme pigment casts that were positive to benzidine and negative for free iron by the Berlin Blue test. At B and B^1 there is disintegration of the basement membrane and a complete disruption of the tubule wall. At B^1 crystals of heme pigment are present. Magnification 84 ×.

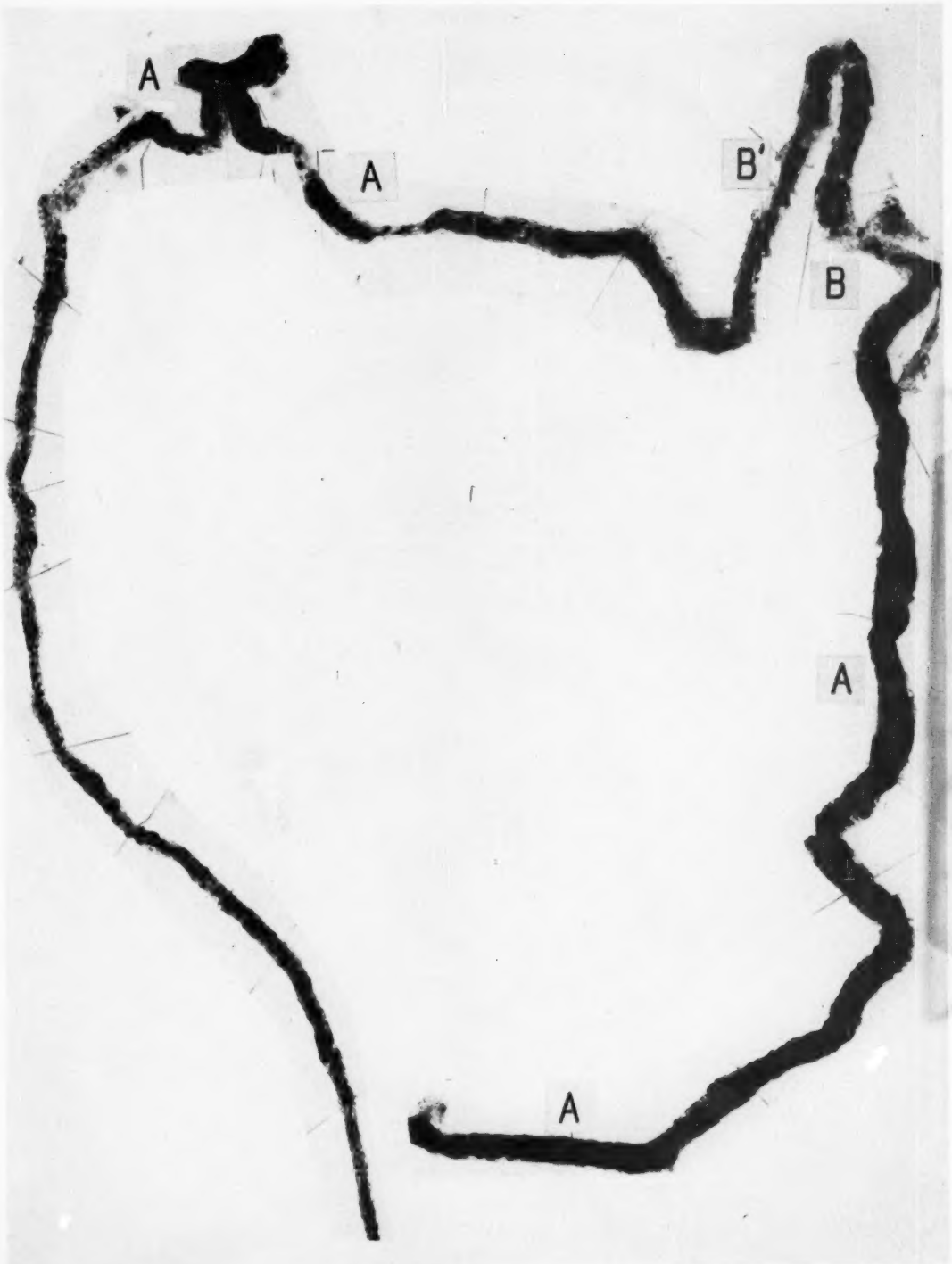

A
A
B'
B
A
A

PLATE 30. DOG K26

Under nembutal anesthesia the right kidney was removed and a serrefine placed on the left renal artery for 60 minutes. The animal remained in good clinical condition and was killed on the fourth day after the operation.

A proximal convolution. The greater part of the tubule is well preserved as is shown by the mitochondrial pattern; at A even the detail of the normal rodlets can be seen. At the arrows this pattern is disturbed and a disintegration of the basement membrane is evident. At B the lesion is fully developed, the tubule is swollen, the basement membrane disintegrated and the renal epithelium necrotic. At C there is a collection of heme pigment impregnated debris; note the continuity and normality of the basement membrane. Magnification 80 ×.

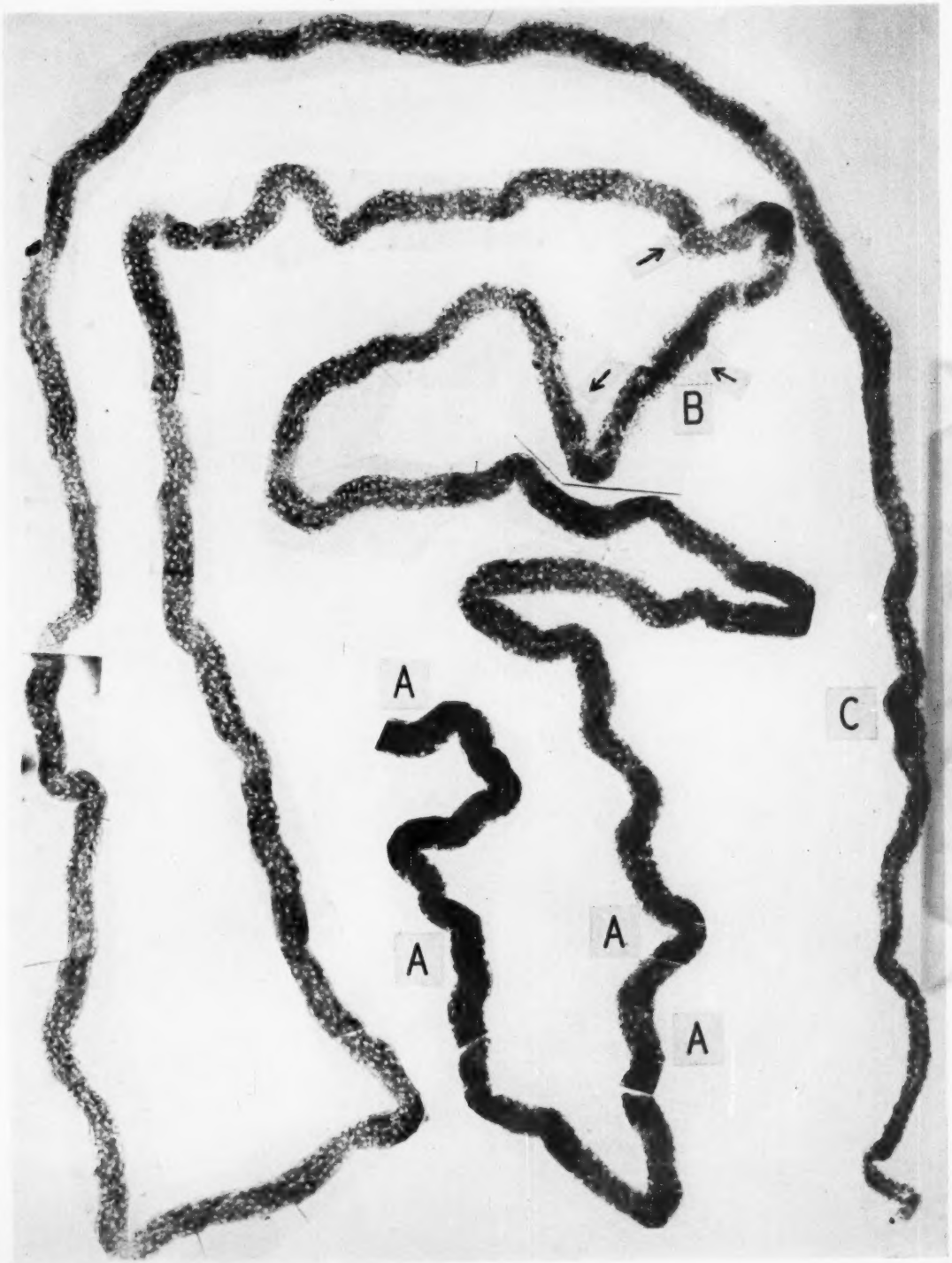

B
A
C
A
A
A
A

PLATE 31. THREE DISTAL CONVOLUTIONS FROM THE SAME KIDNEY (PLATE 30) JOIN TO FORM A COLLECTING TUBULE ON THE LEFT

At A are seen the infrequently mentioned intercalated cells (12) common to all species in this portion of the renal tubule. All of the tubules are essentially normal except at B where an incipient disruption can be seen. At C the distal convolution contains a densely stained heme pigment cast; note that the tubule wall is not disturbed by its presence. Magnification 80 ×.

Insert shows the detail of collecting tubule at A with granular intercalated cells. Magnification 200 ×.

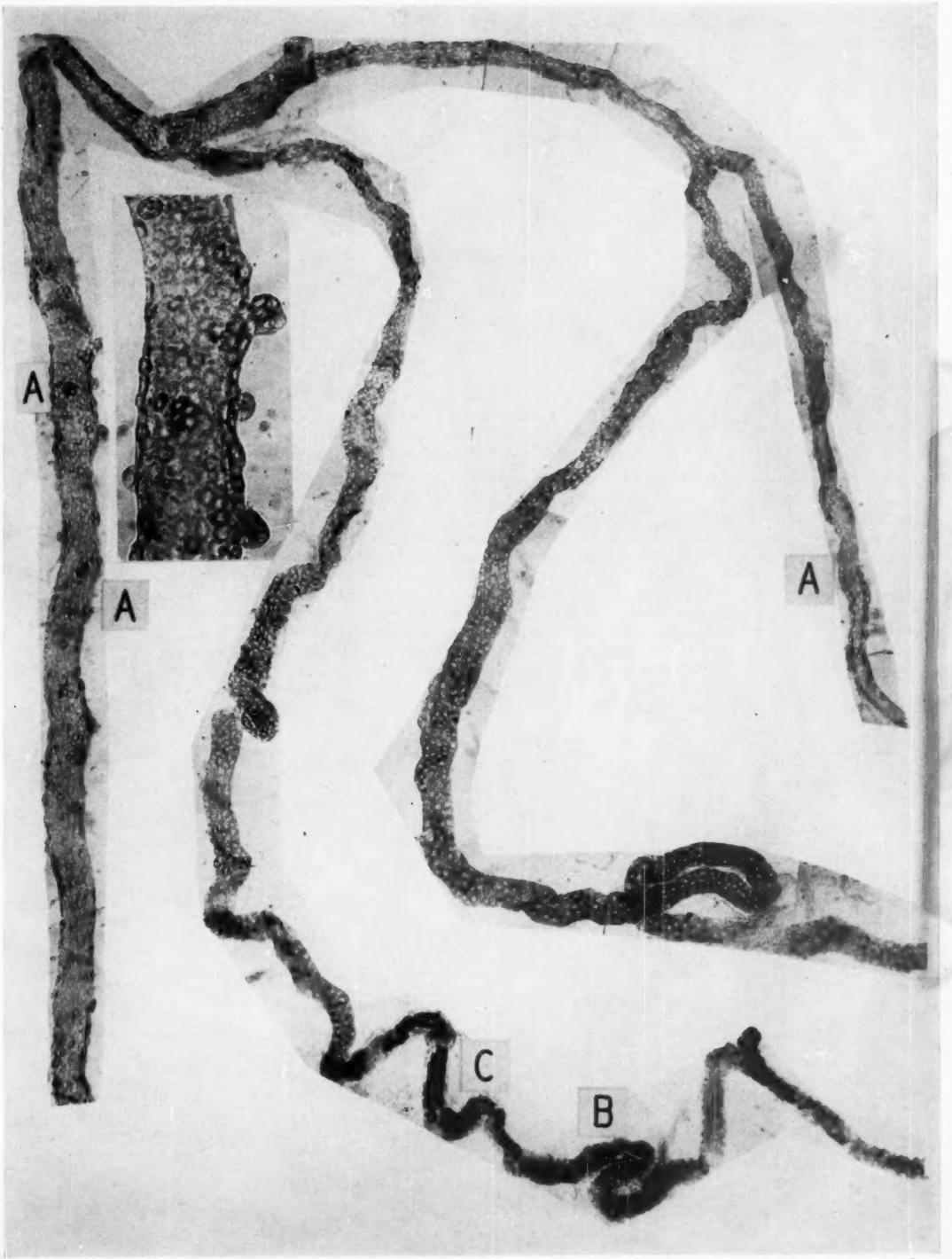

A
A
A
C
B

PLATE 32. DOD K33—THE RESULTS OF EXTREME RENAL ISCHEMIA OF 200 MINUTES DURATION AS MEASURED BY CLEARANCES FOLLOWING TRAUMA UNDER NEMBUTAL ANESTHESIA TO MUSCLE MASSES

A transfusion of the dog's own blood was given at the end of the experiment. Clinical condition of the animal was good on the fourth day. Blood urea 24.8. The animal was then killed.

Proximal convolution and glomerulus. Many nephrons from this kidney were structurally normal; others, perhaps 1/4, resembled the illustration. The mitochondrial pattern of the greater part of the convolution is normal; from B downward the dark staining is due to the normal presence of fat in the convolution. Compare with the similar segment of Plate 28 where both normal fat and disintegration of the basement membrane are present. At A and A^1 are short segments of swollen convolution in which the basement membrane is fragmented, the renal cells necrotic and the tubule disrupted. These are shown at a higher magnification in Plate 33. At the arrows incipient lesions of the same sort are evident. Magnification 41 ×.

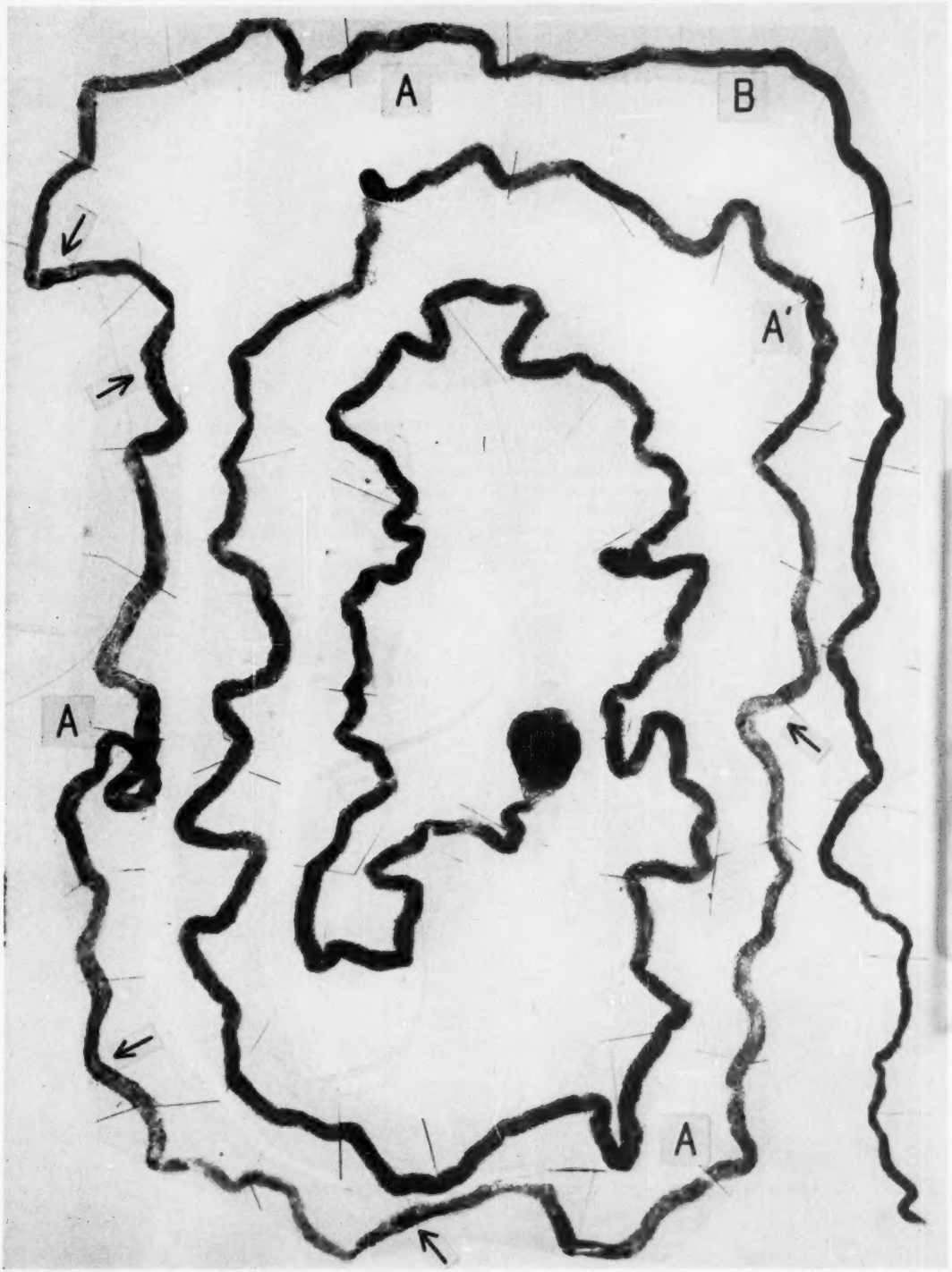

A
B
A'
A
A

PLATE 33. ASCENDING LIMB AND DISTAL CONVOLUTION FROM THE SAME KIDNEY AS IN PLATE 32

Except for the presence of heme casts which have produced no tubular lesion the ascending limb is normal. Its upper portion and the greater part of the distal convolution are distended with heme casts; at A the presence of tubule wall disruption is evidenced by the surrounding adhesions of reactive intertubular tissue. Magnification 45 ×.

Inserts show the detail of the disruptive lesions of the preceding plate at a magnification of 200 ×.

A
A
A
A'

PLATE 34. DOG K32—EXPERIMENTAL PROCEDURE AS IN THE EXPERIMENT OF PLATES *32* AND *33*

A transfusion of the animal's blood was given at the end of the experiment. Before the experiment the blood urea was 10.9; on the fourth day 25.4 and on the seventh, 79.4. The animal, having convulsions, was killed.

Complete proximal convolution and glomerulus. The first 2/3 of the convolution is fairly well preserved, though small areas are visible, as at B^1, where the mitochondrial and nuclear pattern are disturbed as compared to the normality at B. Beginning in the terminal portion of the proximal convolution (A) there is an extensive disintegration of the basement membrane with epithelial necrosis and tubular disruption. Magnification 40×. Lesser disruptions are evident at A^1 and A^2. For detail see following plate at a higher magnification.

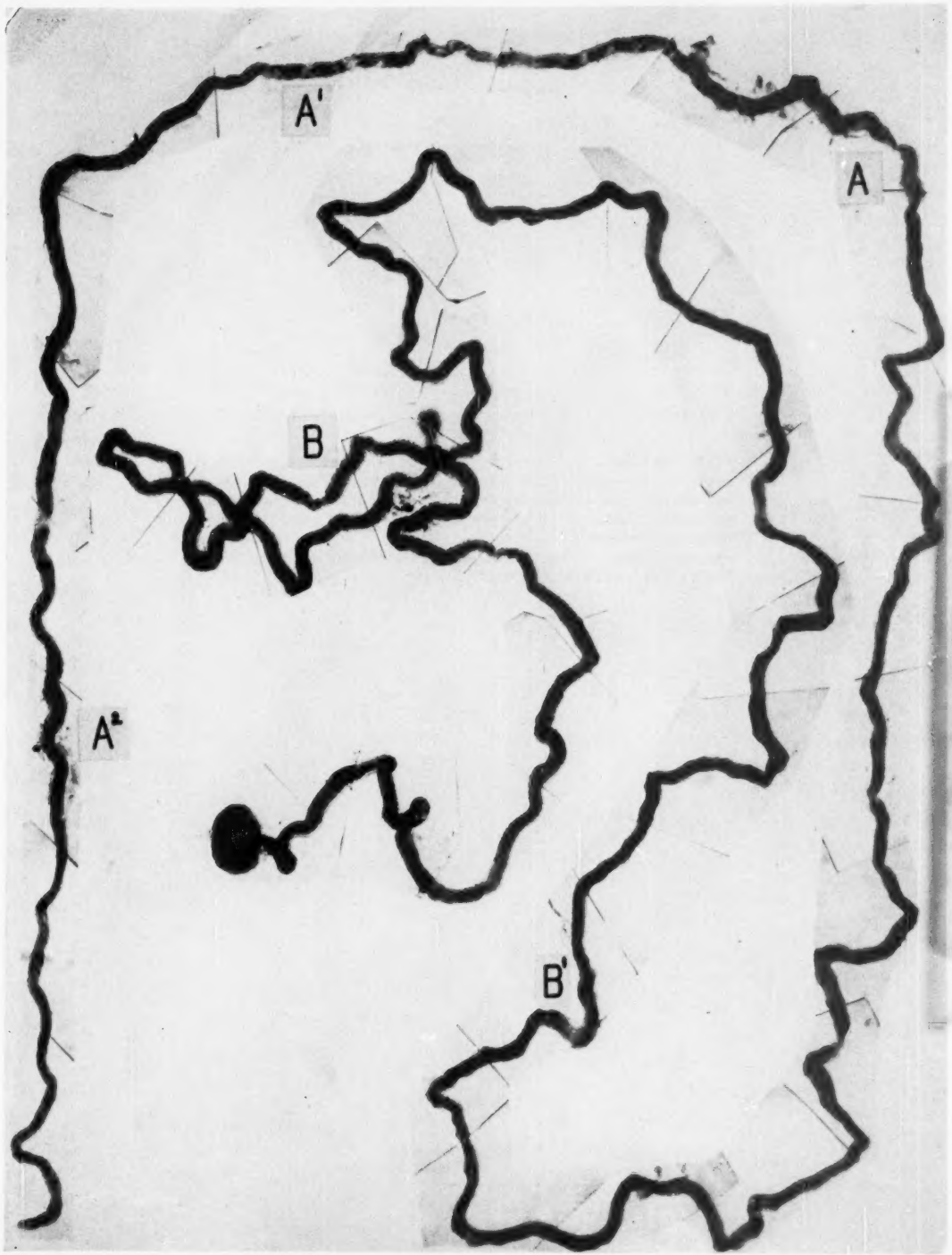

A'
A
B
A²
B'

PLATE 35. A HIGHER MAGNIFICATION OF THE TERMINAL SEGMENT OF PLATE 34

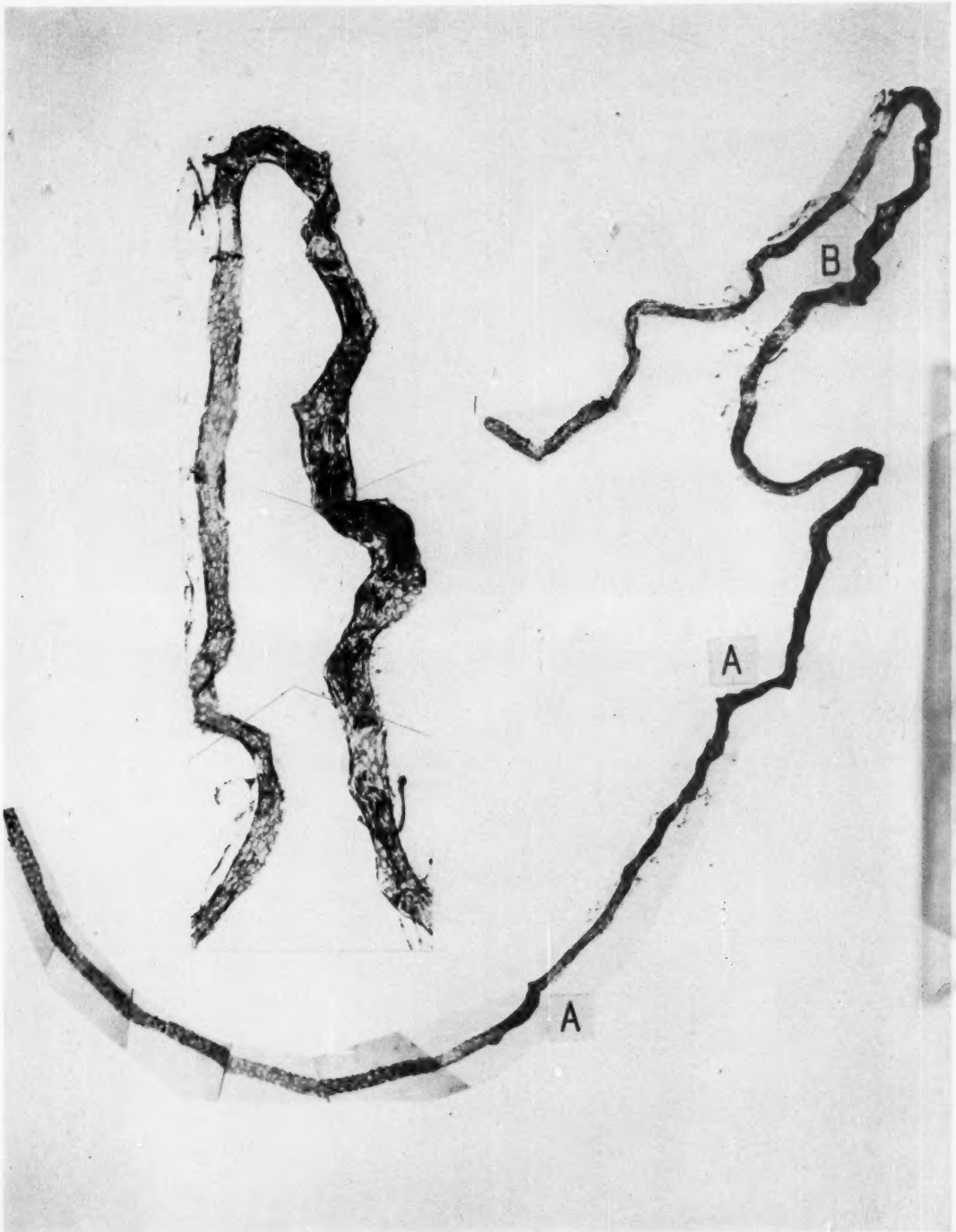
The tubule begins in the center and it will be noted that its first part as it passes upwards is well preserved and the basement membrane intact. At A the epithelial cells are necrotic, the basement membrane disintegrated and the tubule disrupted; a short stretch of better preserved tubule with intact basement membrane follows and at A^1 another disruption. A third disruption, less severe, is seen at A^2 and the tubule following to the thin limb of Henle's loop is again intact. Magnification 82 ×.

A
A²
A¹

PLATE 36. AN ASCENDING LIMB AND DISTAL CONVOLUTION FROM THE SAME KIDNEY AS IN PLATE 34

The ascending limb is entirely normal in its first portion; at A are heme casts. In the distal convolution at B the tubule is swollen, the epithelium reduced to necrotic debris and its wall disrupted. Magnification 45 ×.

Insert shows the disrupted distal convolution at B at a magnification of 100 ×.


B
A
A

Plates 37 to 40

Circulatory Patterns in the Renal Cortex under Normal and Toxic Conditions as Shown by the Schlegel Technique

Magnification of all plates 16×. The experimental animal received under anesthesia 1.0 cc. of 4 per cent thioflavin S intravenously. The kidney was removed in six seconds, split, placed in glycerine and photographed under illumination by Wood's light. The fluorescent dye is held in the walls of the arteries and glomerular tufts. Capillaries show faintly and the veins not at all.

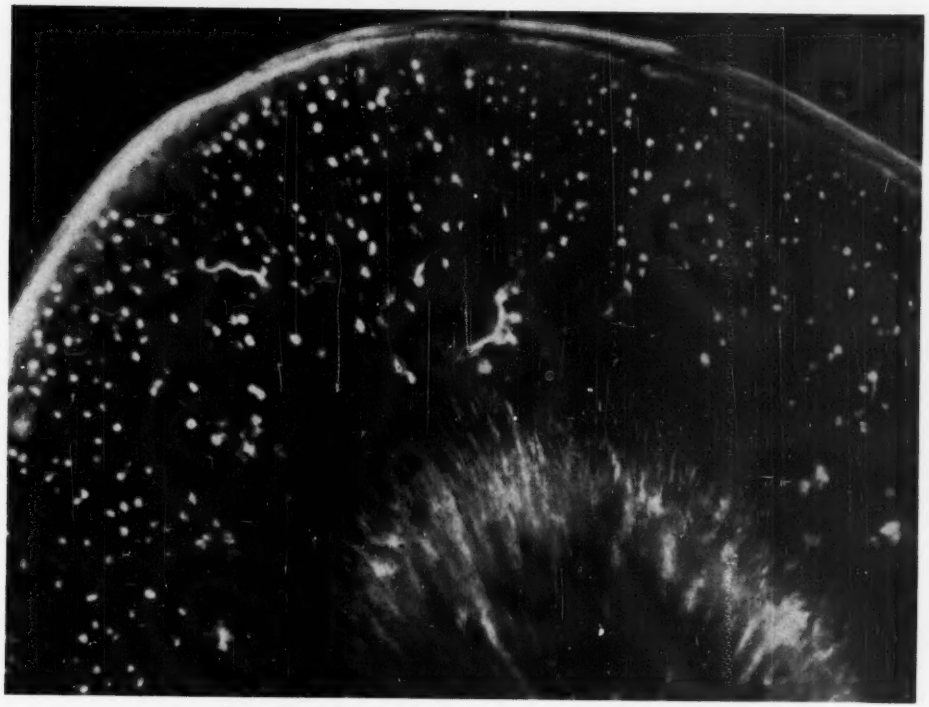
Plate 37

A. The Cortical Circulation in a Normal Rabbit

Note the even distribution of the blood to all glomeruli. The brilliant zone in the outer medulla is due to natural fluorescence of this tissue.

B. The Cortical Circulatory Pattern 19 Hours after the Subcutaneous Injection of 400 mg. of Sodium Potassium Tartrate

A large interlobar artery below with its arcuate branches. There is an irregular area of cortical ischemia where the dye in the blood did not reach glomeruli and intertubular capillaries. To the left an area of more nearly normal blood flow. The disruptive lesions in the nephrons which accompanied the irregular ischemia are seen in Plate 42.



A

B

PLATE 38

A. THE CORTICAL CIRCULATION IN THE KIDNEY OF A RABBIT WHICH HAD BEEN GIVEN 24 MG. OF SUBLIMATE INTRAVENOUSLY 4½ HOURS PREVIOUSLY

There is a marked retardation of blood flow from which only occasional glomeruli received the dye-containing blood.

B. THE OTHER KIDNEY OF THE SAME ANIMAL WHICH WAS ALLOWED TO RECEIVE BLOOD FOR A PERIOD THREE TIMES AS LONG AS THE FORMER

More glomeruli and interlobular arteries have accumulated enough dye to fluoresce, but as compared to the normal pattern of 37A, the irregular patchy ischemia is still evident. Disruptive lesions in the nephrons of another animal which received the same dosage but which lived 18 hours are shown in Plate 42 C and D.

A

B

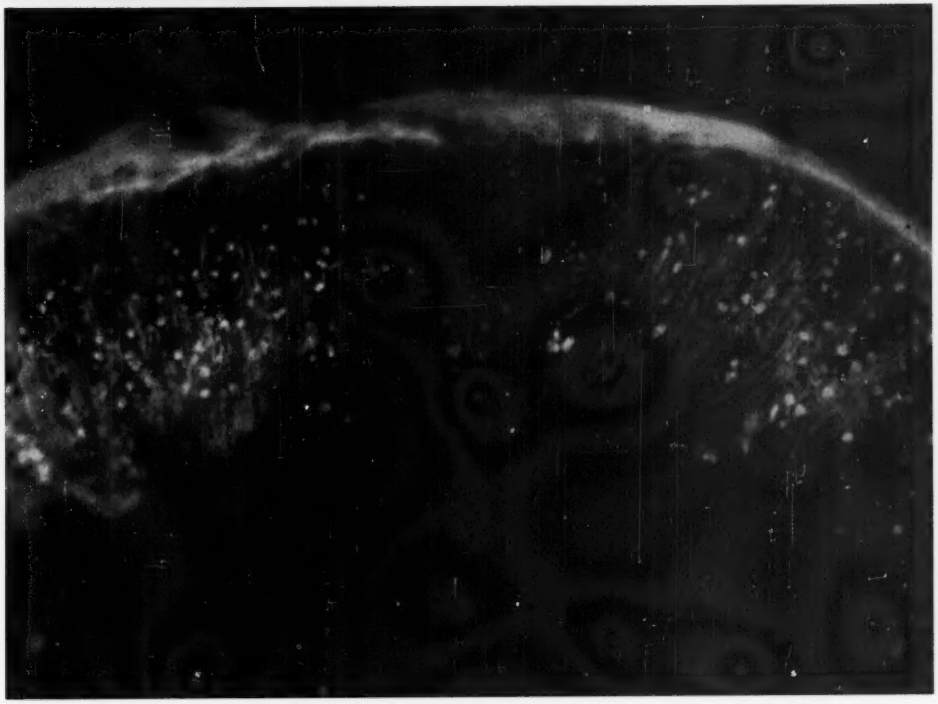
Plate 39

A. The Circulatory Pattern in the Cortex of the Kidney of a Rabbit Which Had Been Given an Intravenous Injection of 40 mg. of Uranyl Nitrate 70 Hours Previously

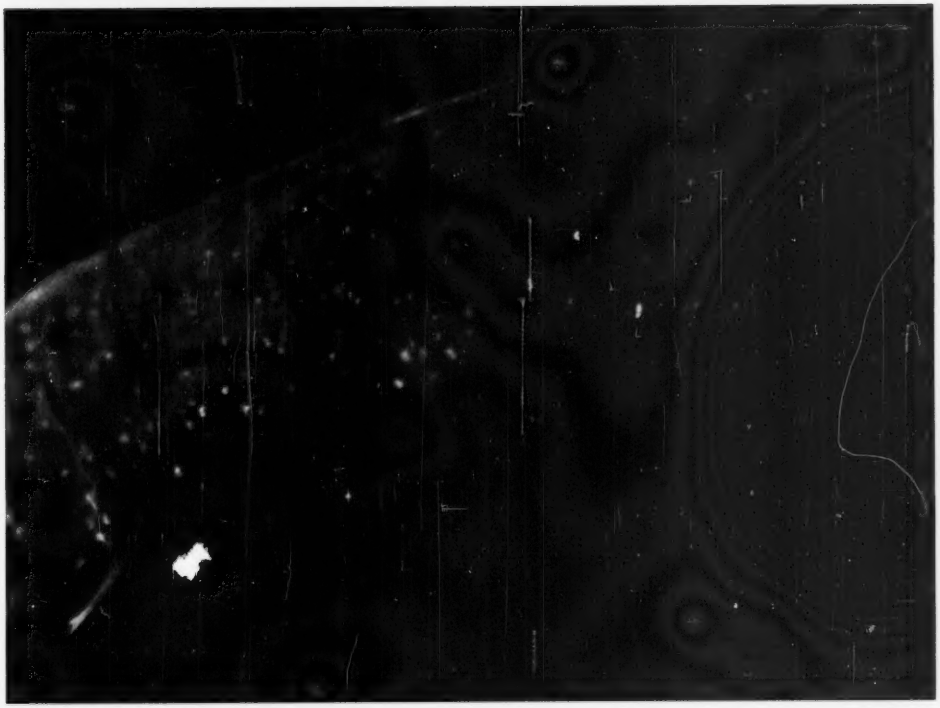
An irregular area of ischemia with few glomeruli is seen. The exposure in this photograph and in Plate 38A was considerably longer than in the other figures hence the background of natural fluorescence of the renal tissue produced a greyish tone which is also somewhat exaggerated by the slight diffusion of the dye through vessel walls. Disruptive lesions in the nephrons of this animal are shown in Plates 42B and 43A.

B. The Cortical Circulatory Pattern in the Kidney of a Rabbit Given 30 mg. of Potassium Bichromate Intravenously 19 Hours Previously

There is a widespread but irregular ischemia of the cortex. The subcortical glomeruli, near the larger arteries, seem better irrigated.



A



B

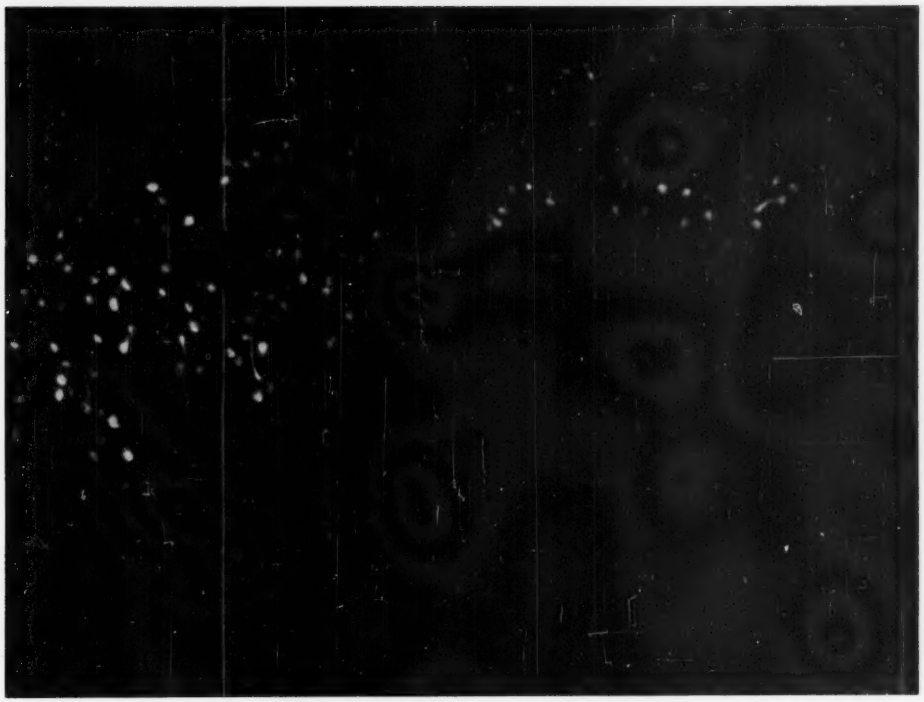
Plate 40

A. Irregularly Patchy Cortical Ischemia in the Kidney of a Rabbit Given 4 cc. of Carbon Tetrachloride on Two Successive Days and Injected with Dye 48 Hours Later

To the left the cortex has received a considerable amount of blood and the glomeruli and small arteries are brightly fluorescent; in the subcortical zone, particularly in the center, they can only dimly be seen. Disruptive tubular lesions from the animal are shown in Plate 43D.

B. The Patchy Cortical Ischemia in the Kidney of a Rabbit Which Had Drunk *ad lib.* Di-Ethylene Glycol over a 24 Hour Period

Disruptive lesions in the nephrons of other rabbits which had drunk the same mixture over periods of five to seven days are shown in Plate 43 B and C.



A

B

PLATE 41. A PROXIMAL CONVOLUTION AND GLOMERULUS FROM A RABBIT POISONED 19 HOURS PREVIOUSLY WITH 400 MG. PER KILO OF SODIUM POTASSIUM TARTRATE

The first few coils of the convolution are fairly normal in their nuclear and mitochondrial pattern but from the arrow distally the epithelium of the entire convolution is necrotic and reduced to cellular debris which fills the intact tube-like basement membrane. At A, however, this structure has disintegrated and typical tubular disruption has resulted. The ischemic pattern of the cortex in this animal is shown in Plate 37B. The disruptive lesions in the ascending limb from another animal which had received the same dosage are shown in Plate 42A. Magnification 82 ×.

A
A
A

PLATE 42. DISRUPTIVE TUBULAR LESIONS FOUND IN ANIMALS THAT HAD RECEIVED VARIOUS TOXIC AGENTS

All of these animals showed the typical nephrotoxic necrosis in the proximal convolutions of all the nephrons along with occasional disruptive lesions in this segment. The occurrence of disruptive, but never nephrotoxic, lesions in other parts of the nephron is illustrated below. *A. Sodium potassium tartrate.* A greatly swollen ascending limb with disintegration of the basement membrane throughout the greater part of its length. At the arrow a more discrete disruption in the distal convolution. *B. Uranyl nitrate.* Basement-membrane disintegration and tubular disruption at the arrows situated at the junction of the ascending limb and the distal convolution. *C and D. Corrosive sublimate.* In C an incipient disruption showing a localized swelling of the tubule and in D another with definite basement membrane rupture. *E. Di-ethylene glycol.* A similar early swelling with disruption in the upper ascending limb. Magnification 80× except B which is 100×.

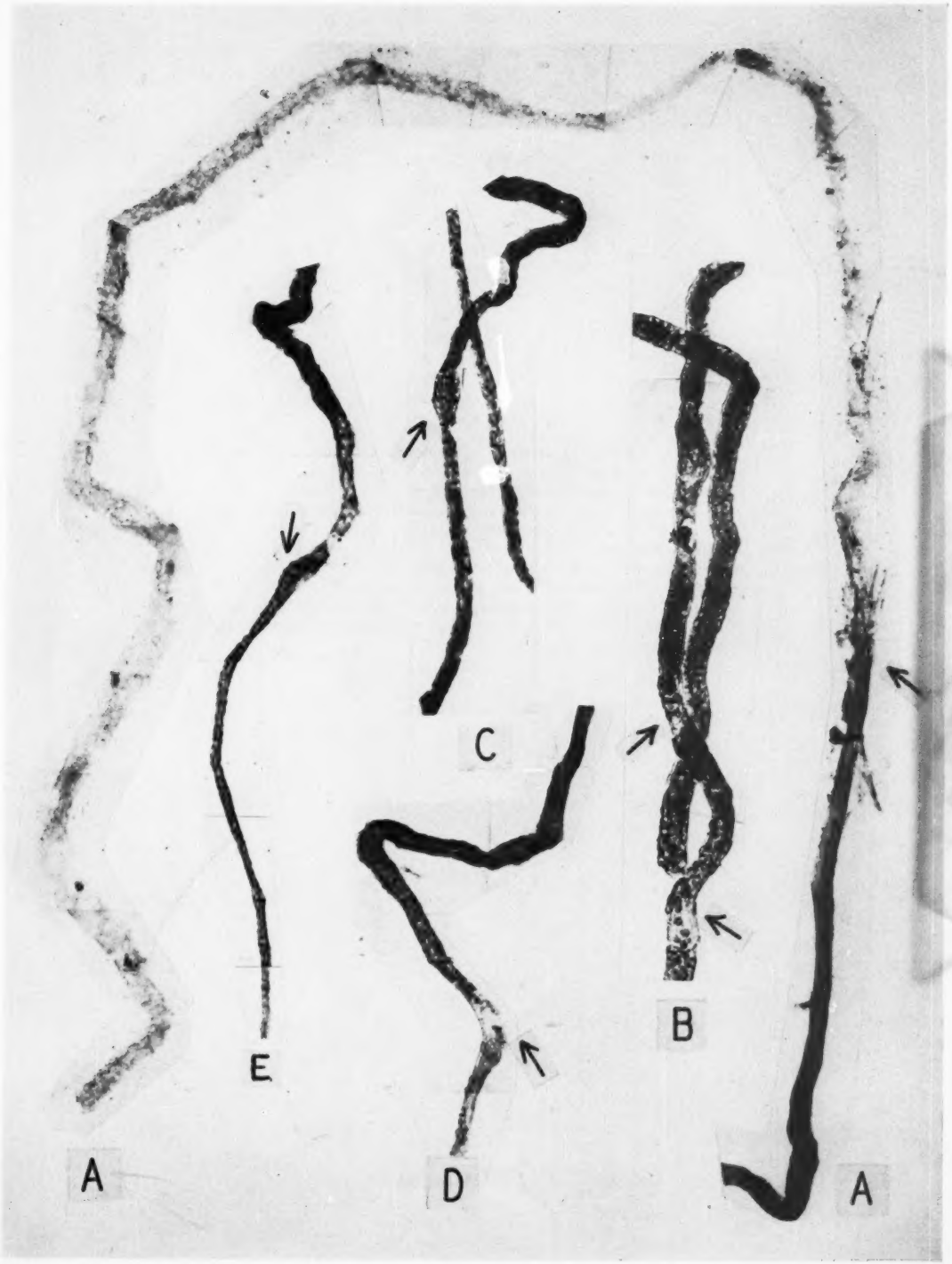

A
E
C
D
B
A

PLATE 43

A. Uranyl nitrate. An ascending limb filled with cellular debris derived from the nephrotoxic damage in its preceding proximal convolution. Disruptive lesions are seen at the arrows. *B and C. Di-ethylene glycol.* At B an incipient lesion at the junction of ascending limb and distal convolution. In C a pair of ascending limbs and distal convolutions are greatly swollen and the basement membrane is almost completely disintegrated. A few necrotic cells persist in the "tubule" lumen and there is a localized disruption above where the two distal convolutions adhere. *D. Carbon tetrachloride.* Two examples of stages of the disruptive lesion in the upper ascending limbs. The dark cells mark the beginning of normally preserved distal convolutions. Magnification 80 ×.

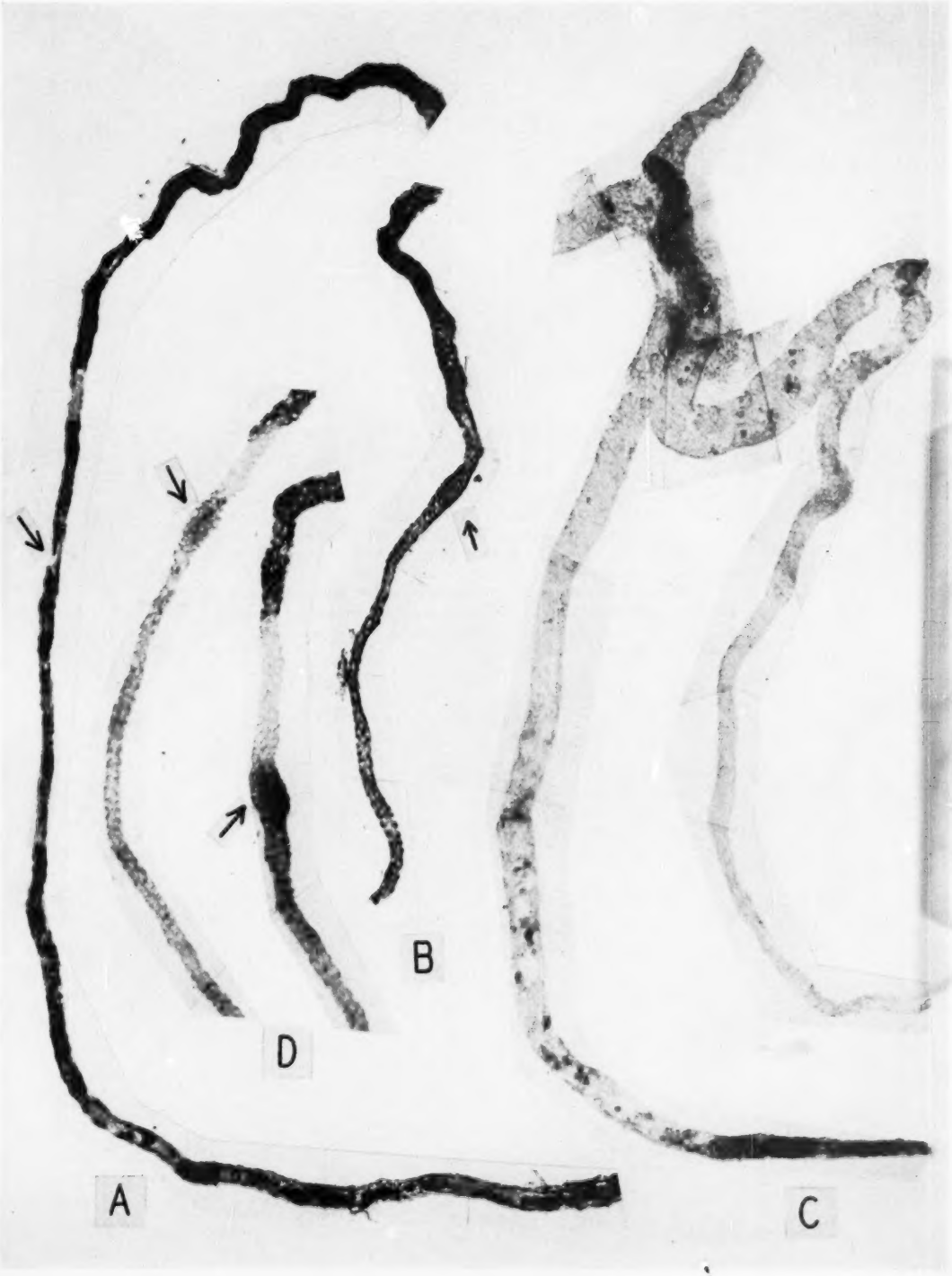
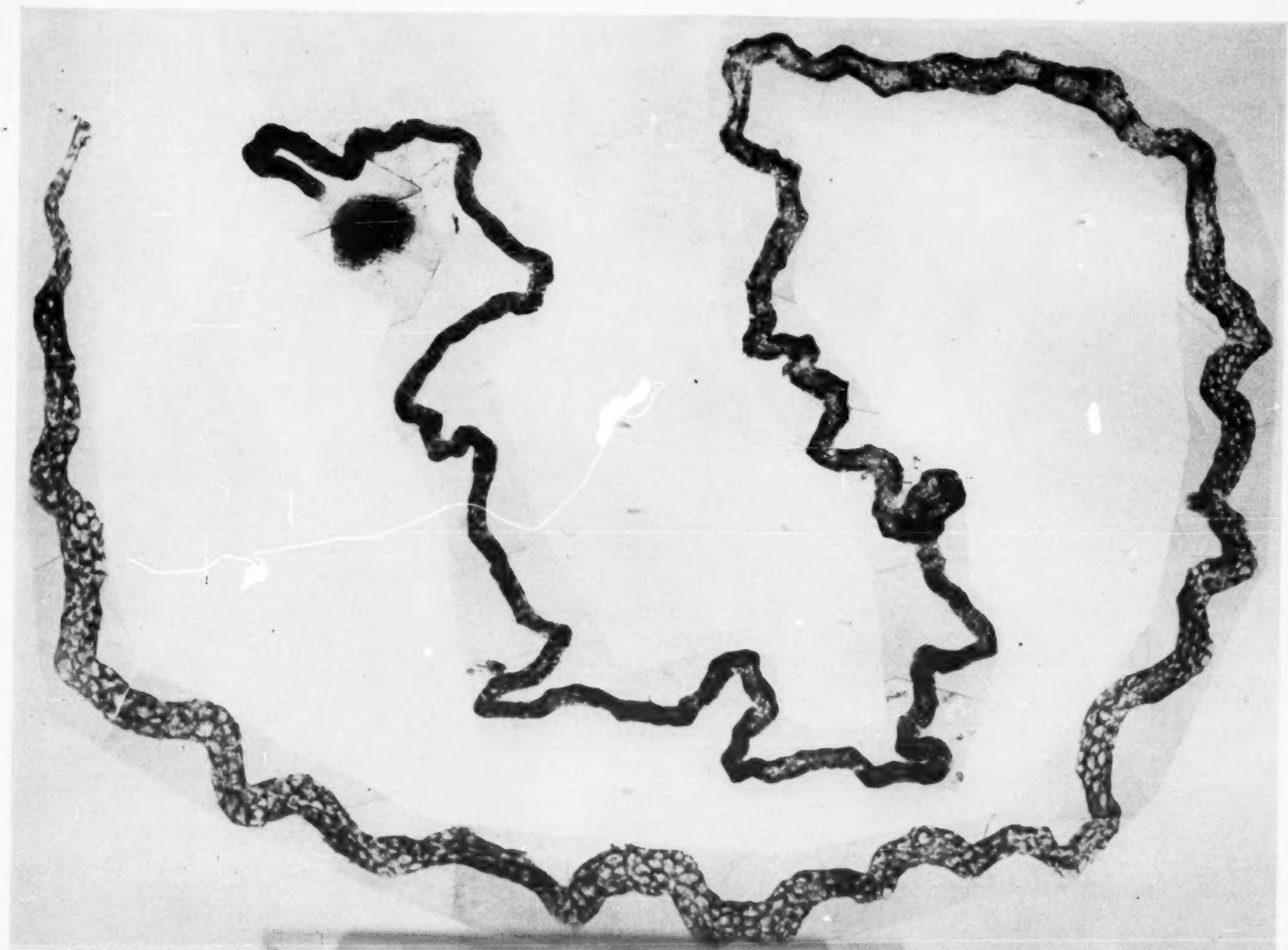
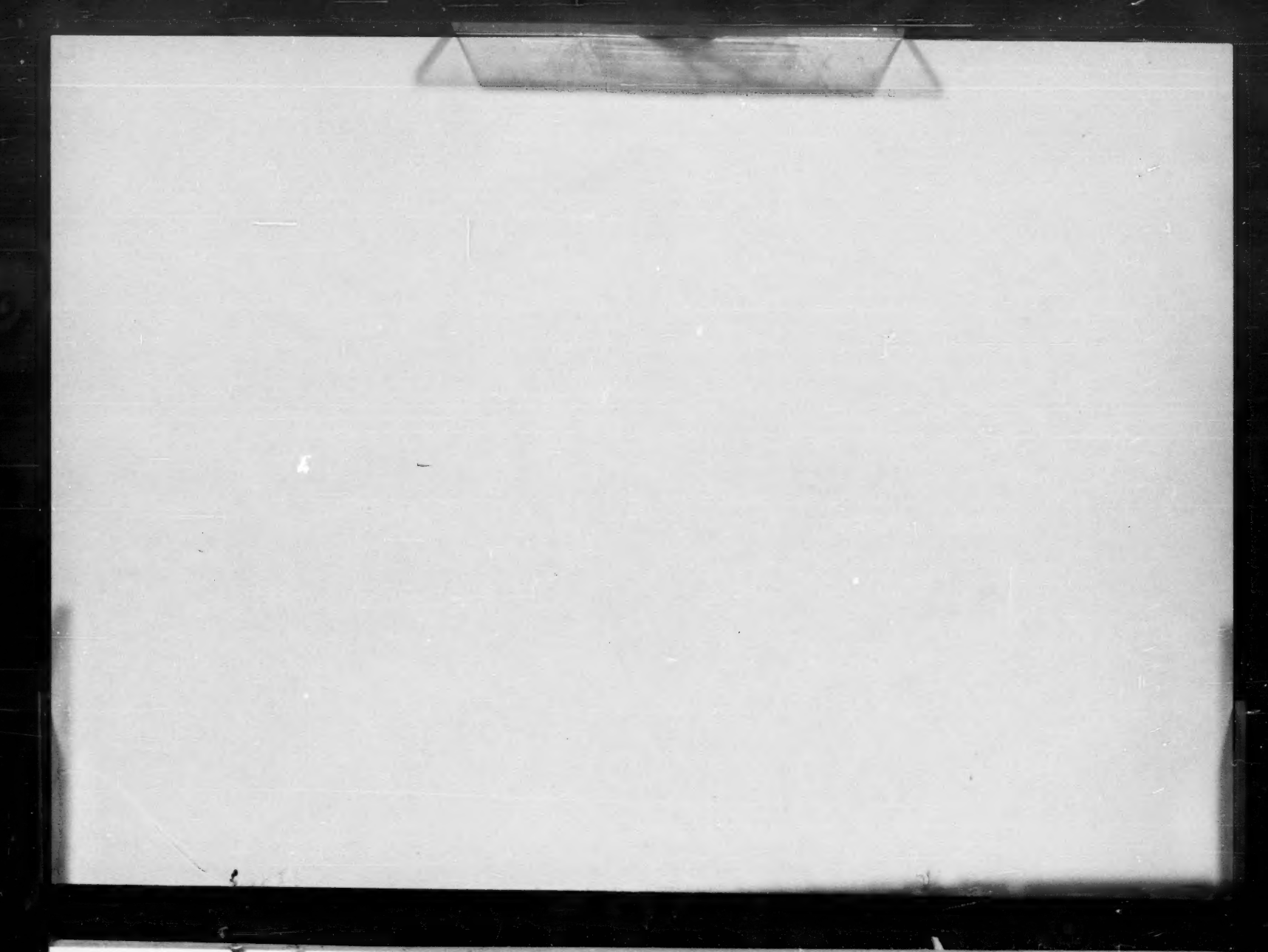

A
B
C
D

PLATE 44. PROXIMAL CONVOLUTION FROM A RABBIT WHICH HAD DRUNK WATER *ad lib.* FOR FOUR DAYS CONTAINING 5 PER CENT DI-ETHYLENE GLYCOL

The localized specific effect of moderate glycol damage is seen in the extreme swelling and vacuolization of the epithelial cells of the terminal half of the proximal convolution. Magnification 82×. Compare with the appearance in Figures 3 and 4 and with Plate 22 which shows more severe damage in the human kidney.

The disruptive damage in ascending limbs in this and other similarly poisoned animals is seen in Plate 42E in its incipient form and fully developed in Plate 43C. The pattern of cortical ischemia that accompanies glycol poisoning is shown in Plate 40B.





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PART 2

The Journal of Clinical Investigation

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Contents

Venous Congestion of the Extremities in Relation to Blood Volume Determinations and to Mixing Curves of Carbon Monoxide and T-1824 in Normal Human Subjects. ELLEN BROWN, JAMES HOPPER, JR., JOHN J. SAMPSON, AND CHARLES MUDRICK 1441

Quantitative Antibody Studies in Man. I. The Effect of Adrenal Insufficiency and of Cortisone on the Level of Circulating Antibodies. DANIEL L. LARSON AND LOIS J. TOMLINSON 1451

The Renal Response to Intravenous Injection of Sodium Chloride Solutions in Man. BETTY CRAWFORD AND HENRY LUDEMANN 1456

The Velocity of Distribution of Sucrose between Plasma and Interstitial Fluid, with Reference to the Use of Sucrose for the Measurement of Extracellular Fluid in Man. NORMAN DEANE, GEORGE E. SCHREINER, AND JAMES S. ROBERTSON 1463

Intracellular Water in Man. NORMAN DEANE 1469

Total Red Cell Volume, Plasma Volume and Sodium Space in Congestive Heart Failure. THEODORE C. PRENTICE, NATHANIEL I. BERLIN, GRACE M. HYDE, ROBERT J. PARSONS, JOHN H. LAWRENCE, AND SHIRLEY PORT 1471

Studies in Fat Metabolism. I. The Colorimetric Determination of Ketone Bodies in Biological Fluids. GEORGE D. MICHAELS, SHELDON MARGEN, GERALD LIEBERT, AND LAURANCE W. KINSELL 1483

Studies in Fat Metabolism. II. Evaluation of the Effect of Testosterone Propionate upon Ketone, Carbohydrate, and Protein Metabolism in a Patient with Diabetes Mellitus Complicated by Thyrotoxicosis. LAURANCE W. KINSELL, SHELDON MARGEN, GEORGE D. MICHAELS, AND DAVID P. MCCALLIE 1486

Studies in Fat Metabolism. III. The Effect of ACTH, of Cortisone, and of Other Steroid Compounds upon Fasting-Induced Hyperketonemia and Ketonuria. LAURANCE W. KINSELL, SHELDON MARGEN, GEORGE D. MICHAELS, ROBERT REISS, ROBERT FRANTZ, AND JOHN CARBONE 1491

The Effects of Adrenaline upon Renal Function and Electrolyte Excretion. W. E. JACOBSON, J. F. HAMMARSTEN, AND B. I. HELLER 1503

A Quantitative Relationship between the Water Content of Skin and Inflammation Produced by Chemical Irritants. H. VINIK WEISS, VINTON E. SILER, AND PETER R. BUECHLER 1507

Ferrokinetics in Normal Persons and in Patients Having Various Erythropoietic Disorders. REX L. HUFF, PAUL J. ELMLINGER, JOSEPH F. GARCIA, JOHN M. ODA, MARION C. COCKRELL, AND JOHN H. LAWRENCE 1512

The Effect of Adrenocorticotrophic Hormone (ACTH) on Cerebral Blood Flow and Metabolism. JAMES F. SCHIEVE, PERITZ SCHEINBERG, AND WILLIAM P. WILSON 1527

Treatment of Nephrosis with Pituitary Adrenocorticotrophin. JOHN A. LUETSCHER, JR., QUENTIN B. DEMING, AND BEN B. JOHNSON 1530

Hemagglutinins and Hemolysins for Erythrocytes Sensitized with Tuberculin in Pulmonary Tuberculosis. WENDELL H. HALL AND ROBERT E. MANION 1542

Index to Volume XXX 1547

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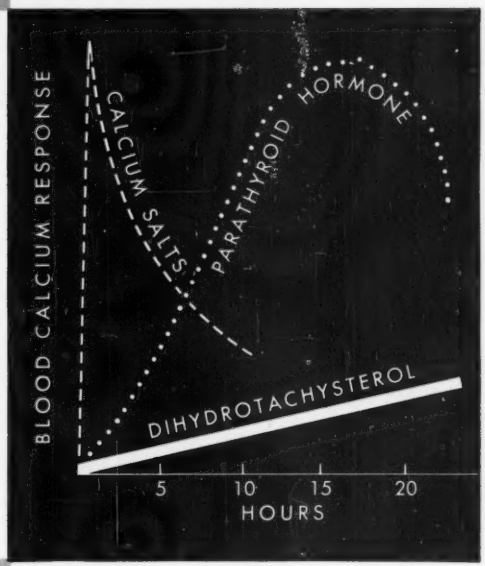

Blood calcium response to various forms of therapy. Note slow, steady rise following dihydrotachysterol orally.*

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*Grollman, A.: Essentials of Endocrinology. Philadelphia, J. B. Lippincott Co., 1947, 2d ed., p. 267,269.

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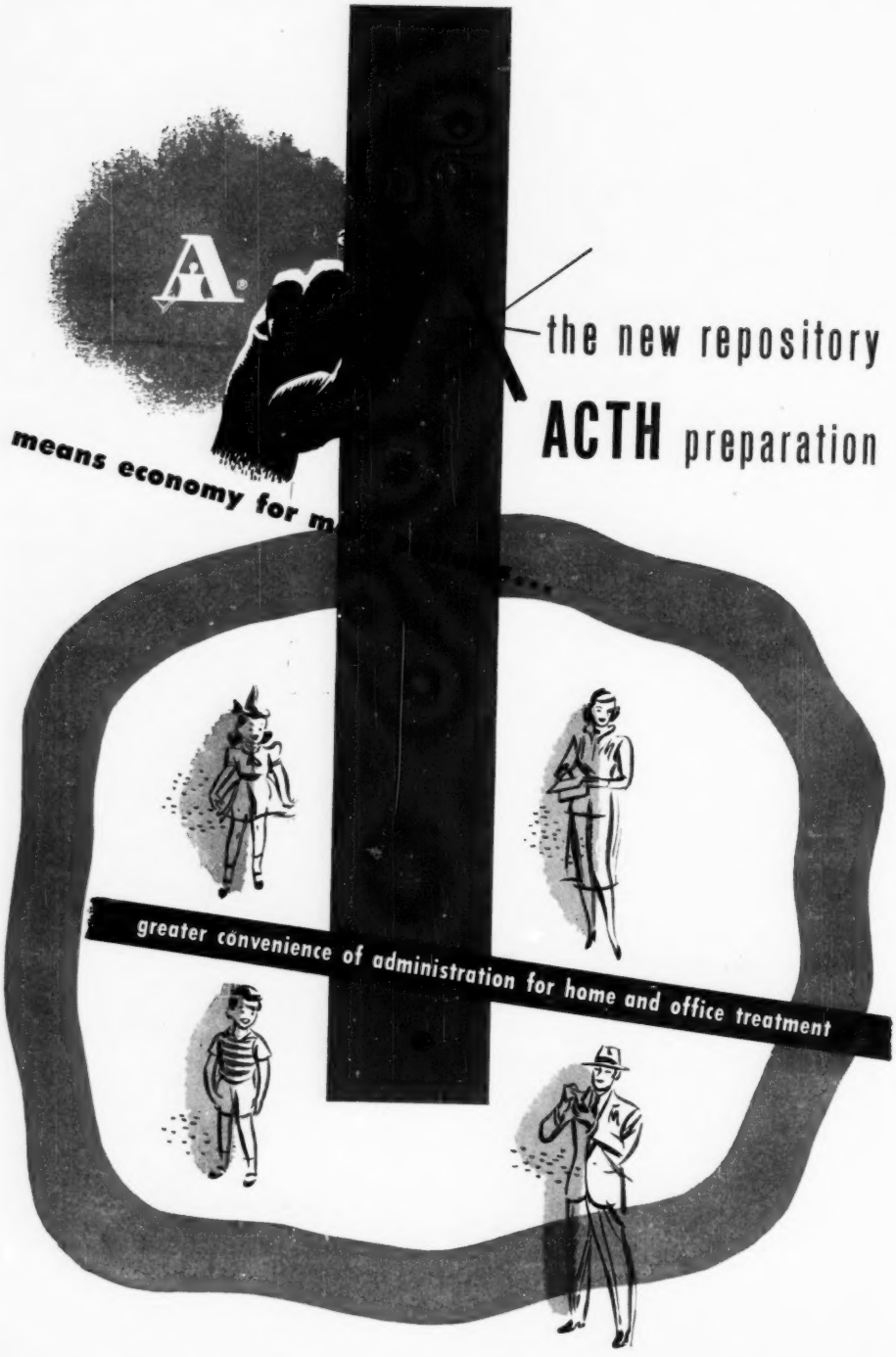
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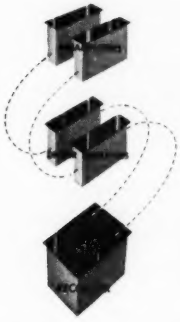

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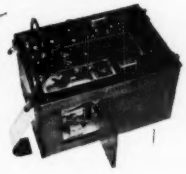

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VENOUS CONGESTION OF THE EXTREMITIES IN RELATION TO BLOOD VOLUME DETERMINATIONS AND TO MIXING CURVES OF CARBON MONOXIDE AND T–1824 IN NORMAL HUMAN SUBJECTS [1]

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(From the Division of Medicine, University of California School of Medicine, San Francisco, California)

(Submitted for publication March 26, 1951; accepted August 20, 1951)

Investigations of circulatory problems often involve measurement of the blood volume in clinical conditions which may be characterized by important grades of stasis or venous congestion, such as pregnancy, heart failure, and shock.

As yet, no serious efforts have been made to determine the limitations of the common methods for measuring blood volume under such circumstances, although it has been claimed that delayed mixing of dyes may occur during postural stasis (*1*) and heart failure (*2*) and that serious leakage of protein-bound dyes may result from splanchnic congestion (*3*).

In the studies to be described, congestion was imposed on the limbs of healthy human subjects by pneumatic cuffs in such a way as to involve as much as one-fourth of the total blood volume at pressures near diastolic arterial levels. It will be shown that even such massive pooling of blood in the veins does not affect the measurement of total blood volume by a carbon monoxide method. Curves representing time-concentration relationships of carbon monoxide (CO) and the dye T-1824 in venous blood were only slightly modified by the presence of congestion, but showed that in the presence of massive congestion the use of sampling periods shorter than 15 to 20 minutes may lead to falsely low estimations of blood volume.

The results tend to support the modern concept, as contrasted to certain earlier, less dynamic views, concerning the nature of blood volume reservoirs (*4, 5*).

METHODS

The carbon monoxide method used has been described in detail elsewhere (*6, 7*). Essentially, it involves the introduction of a carefully measured volume of pure CO into a small closed system, through which the subject rebreathes for the duration of the experiment, oxygen being added as required. Samples of venous blood taken before, and at an interval of 20 minutes or longer after delivery of the gas are analyzed in triplicate for CO by a chemical technique utilizing palladium chloride (*6*).

The total CO-available volume is calculated by the following formula:

$$\frac{V_d \times P}{C_f - C_i} \times 100,$$

where V_d is the apparent volume of CO which was delivered from the gas burette to the rebreathing system corrected to standard conditions of pressure and temperature, P is a correction factor for purity of the gas, and C_i and C_f the concentrations of CO in whole venous blood samples respectively, before, and at an interval of 20 minutes (or longer) after delivery of the gas.

For each sample of blood subjected to analysis for CO, the packed cell volume was determined, so that the total volume of erythrocytes could be calculated (see Figures 2 and 4). The anticoagulant was a mixture of ammonium and potassium oxalates (*8*). Wintrobe hematocrit tubes of 1 ml. capacity were filled in duplicate and centrifuged for 30 minutes at 3,000 r.p.m. Hematocrit values were not corrected for "trapped plasma" (*9*) because these experiments were concerned only with relative changes of volume, not with absolute volumes of cells or of whole blood.

By multiplying values for total CO-available volume as obtained from analysis and the hematocrits of the corresponding blood samples, total erythrocyte volumes were calculated. The latter quantity reflects existing physiological conditions more accurately than does total CO-available volume because it is not subject to errors of unknown magnitude which may result from differences between the hematocrit of peripheral venous blood and "total body hematocrit" at the time of sampling (*10*).

The major errors to be considered in using this method relate to (*1*) purity of the stock gas, (*2*) residual CO in the lung-rebreathing space and leakage from this system, (*3*) loss of CO to extravascular pigment stores during the interval between delivery of the gas and sampling, and (*4*) accuracy of chemical determination of CO in blood before and after delivery of the gas.

[1] This work was supported by a grant from the Christine Breon Fund for Medical Research.

The purity of the stock CO was measured by absorption with Winkler's cuprous chloride solution using a Scholander syringe, assuming the contaminating gas to be room air. Each blood volume was appropriately corrected in terms of this measurement. Suitable precautions were taken to assure airtight connections in the rebreathing system and delivery burette. No correction was made for residual CO in the lung-rebreathing space because errors arising from this source amount to less than 1 per cent of the final blood volume when a small rebreathing system is used (6). This is in contrast to the major errors involved with larger breathing systems (11).

It is generally accepted that estimates of absolute circulating red cell volume obtained with CO methods may be slightly larger than the true volumes because of CO loss to extravascular pigment stores (12). Any such losses which may be significant, however, must take place during the earliest phases of mixing because curves obtained during prolonged rebreathing show levels of CO in the blood which are constant within the limits of analytical error for periods of an hour or more following the initial 15 minutes of mixing (6, 7, 13–15). Examples of such curves (see Figure 1) demonstrate the suitability of this method for long experiments of the type reported here, where relative changes, rather than absolute values for blood volume, are being studied. The CO methods have the unique advantage of safety when repeated measurements at frequent intervals are required, as in these experiments.

With the method used, the absolute magnitude of possible error introduced by the chemical analysis varies directly with the magnitude of the blood volume, because the quantity of gas delivered to the subject is ordinarily the same (approximately 100 ml.) in each experiment. In a series of 134 determinations of blood volume performed under similar conditions and by the same personnel as the experiments described here, the standard error of the difference in CO content between any pair of samples (C_f–C_i) was found to be 0.026 volume per cent (7). From this it may be calculated that, in terms of blood volume, 95 per cent of pairs of analyses would be expected to give values within ± 200 ml. of the largest blood volume (6100 ml.) and within ± 130 ml. of the smallest blood volume (3760 ml.) observed in the course of these experiments.

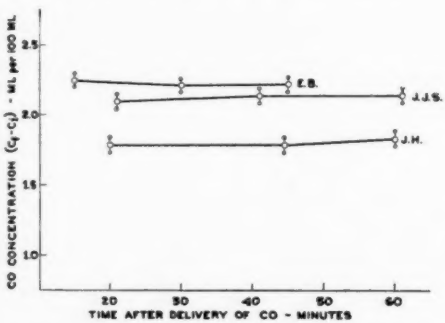

FIG. 1. CONCENTRATIONS OF CO IN VENOUS BLOOD (C_f–C_i) AT VARIOUS INTERVALS AFTER INTRODUCTION OF THE GAS DURING THREE EXPERIMENTS IN WHICH THE SUBJECTS WERE AT REST AND WERE CONNECTED CONTINUOUSLY TO THE SMALL REBREATHING SYSTEM

Small circles represent the magnitude of expected analytic error (plus and minus twice the standard error of C_f–C_i).

PROCEDURES

The subjects rested for 30 minutes in a recumbent position before any procedure was begun. Room temperature was in the neighborhood of 20° C. and subjects were kept warm by means of blankets and electric heating pads on the feet, as required for comfort. One or, at the most, two venipunctures were sufficient for the collection of all blood samples. The 18-gauge needle inserted in an antecubital vein was kept open by means of a constant, very slow infusion of 0.9 per cent saline. Blood was allowed to flow freely through the needle for at least 15 seconds before any sample was drawn.

Venous congestion or arterial occlusion was produced by inflation of pneumatic cuffs, 15 cm. in width, surrounding each thigh just below the groin, and a cuff, 10 cm. in width, placed high on the left arm. The three cuffs communicated with an air reservoir so that they could be completely inflated to the desired pressures within 10 seconds.

Each of three healthy subjects, two male and one female, was subjected to the following experiments.

Procedure 1

The experiment was designed to determine whether CO-available volume is significantly smaller in the presence of massive venous congestion than following release of the obstruction. The cuffs surrounding the three limbs were first inflated to between 0 and 10 mm. Hg below diastolic arterial pressure. After 10 minutes, a blood sample was drawn, the subject began to breathe into the closed system, and the CO was delivered. After 20 minutes of rebreathing during which congestion continued, a blood sample was drawn for determination of the CO-available volume during congestion. The cuffs were then released and blood samples drawn at approximately 10-minute intervals. Rebreathing was not stopped until the last sample had been collected, 18 to 44 minutes after the cuffs were released.

Procedure 2

The purpose of this procedure was to determine whether curves representing time-concentration relationships of CO in venous blood might show significant differences in contour when made with congestion and without. For each subject, a pair of experiments was carried out in which CO concentration was determined at frequent in-

tervals following administration of the gas; first, during venous congestion which was of similar intensity to that used in Procedure 1, and, on another day, under resting conditions. Samples of venous blood were collected at intervals of 30 to 60 seconds for the first three minutes and thereafter less frequently for a total of 20 minutes.

Procedure 3

For purposes of comparison, venous time-concentration curves were made for each subject with the dye T-1824, but only during congestion. It was thought that similar curves made without congestion using dye would not be of sufficient value to warrant repeated injections of T-1824 into subjects often exposed to such procedures. After 10 minutes of congestion, at pressures similar to those used in Procedures 1 and 2, and while the cuffs were still inflated, 5 ml. of an 0.5 per cent solution of T-1824 were injected intravenously. Samples of venous blood were collected at intervals of 30 seconds for the first three minutes and at four, six, nine, 12, 15, and 20 minutes after delivery of the dye. Concentrations of dye in the serum were measured with the Evelyn photoelectric colorimeter (16).

Procedure 4

This was carried out in order to measure the approximate volume of blood which had been present in the congested limbs under the conditions of the foregoing experiments. The procedure was as follows: The cuffs were inflated to a pressure which was the same for each subject as that used in Procedure 1. After congestion had continued at this pressure for 10 minutes, the pressure in the cuffs was increased suddenly to 200 mm. Hg or higher. A blood sample was then obtained and the CO delivered. After rebreathing had continued for 20 minutes, a sample of blood was taken for estimation of CO-available volume of the body, exclusive of those portions separated from the circulation by means of the arterial tourniquets. The cuffs were then released and rebreathing and intermittent sampling continued for periods of 6½ to 30 minutes.

Procedure 5

In order to determine the volume of blood contained in the three extremities of each subject when not congested, thus permitting calculation of the relative increase of vascular volume caused by congestion, a procedure was carried out which was identical to Procedure 4, except that arterial occlusion was not preceded by congestion.

RESULTS

1. *CO-available volume during and immediately after congestion*

Figure 2 shows the results obtained with two subjects when CO-available volume was determined during venous congestion and after release of the congesting cuffs (Procedure 1). The congesting pressure used for J. J. S. was 85 mm. Hg, and for E. B., 65 mm. Hg.

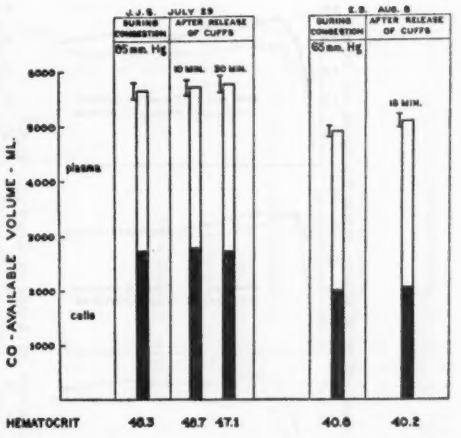

FIG. 2. CO-AVAILABLE VOLUME, MEASURED DURING CONGESTION OF THREE EXTREMITIES BY PNEUMATIC CUFFS, AND AFTER RELEASE OF THE CUFFS, IN TWO SUBJECTS

Cuff pressures, which are indicated at top of chart, were just below diastolic arterial pressure. Blood samples for determination of congested volume were taken 20 minutes after delivery of the gas, and after cuffs had been in place for 30 minutes. Subjects rebreathed through a closed system throughout. Here, and in Figure 4, full columns represent total CO-available volume and shaded portions the total cell mass, calculated from hematocrits shown below. Small bars beside columns represent the range of total CO-available volumes equivalent to plus and minus two standard errors of the CO concentration (C_f–C_i).

In both the experiments illustrated, the total erythrocyte mass measured during congestion was the same as that found after the cuffs were released. The slight increase of plasma volume which occurred in each subject following release of the cuffs, and which was reflected by a falling hematocrit, could be attributed to reabsorption of extracellular fluid which had been filtered from the capillaries of the limbs during the previous 30 minutes of massive congestion. Although for this reason total CO-available volume tended to be slightly greater after release of the cuffs than during congestion, the difference was within the limits of error of the method, as shown by the chart.

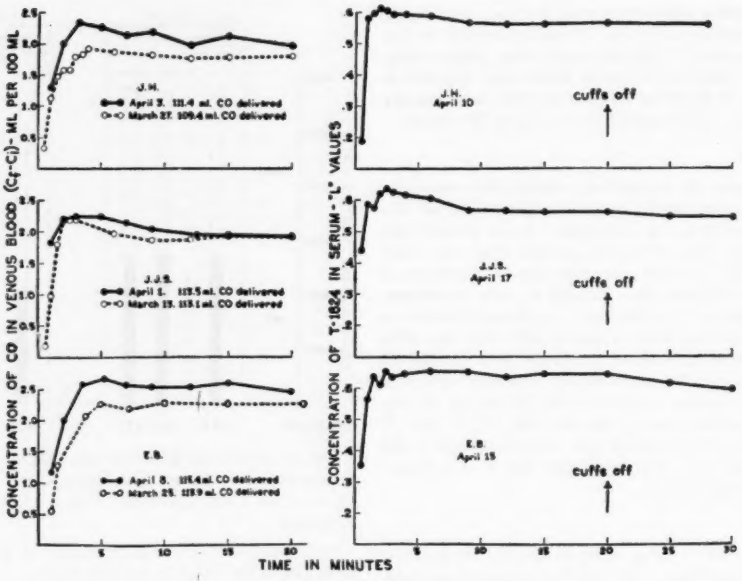
CONCENTRATION OF CO IN VENOUS BLOOD (Cf-Ci) - ML PER 100 ML
CONCENTRATION OF T-1824 IN SERUM - "L" VALUES
J. H.
April 3. 111.4 ml. CO delivered
March 27. 109.4 ml. CO delivered
J. J. S.
April 1. 113.5 ml. CO delivered
March 15. 113.1 ml. CO delivered
E. B.
April 8. 115.4 ml. CO delivered
March 25. 115.9 ml. CO delivered
J. H.
April 10
cuffs off
J. J. S.
April 17
cuffs off
E. B.
April 15
cuffs off
TIME IN MINUTES

FIG. 3.

Left. Time-concentration curves of CO under resting conditions (open circles and dotted lines) and during congestion of three extremities (solid circles and solid lines).

Right. Time-concentration curves for T-1824 during congestion of three extremities and following release of cuffs. Dye-concentrations are expressed as the equivalent "L" values (14).

2. Time-concentration curves of CO and T-1824 with and without congestion

The time-concentration curves of CO and T-1824 which were obtained by Procedures 2 and 3 are shown in Figure 3. At the left side of the figure are seen the CO curves obtained for each subject (*a*) during congestion of three extremities at pressures just below diastolic arterial pressure (solid circles and solid lines) and (*b*) at a different session, without congestion (open circles and broken lines). The final concentration of CO after 20 minutes of rebreathing was not the same in each pair of experiments because the quantity of gas delivered was not exactly the same nor was the blood volume of each subject necessarily the same on each occasion.

All the curves, whether obtained with or without congestion, show irregularities and peaks during the first 10 minutes and become essentially flat after 15 minutes of rebreathing. In all three pairs of experiments, the curves obtained during congestion had more conspicuous peaks and remained higher than the final level for longer times than did the curves obtained without congestion. This is seen by direct inspection in the case of subject J. J. S. (Figure 3, center, left) where the final concentrations of CO happened to be the same in both experiments. It can be shown to be true also for the other two subjects where, even if corrections are made to bring the curves to the same final concentration, the curves during congestion still lie well above the curves obtained without congestion during the first 10 to 12 minutes of rebreathing.

To the right in Figure 3 are shown the time-concentration curves for T-1824 which were made for the three subjects during congestion. In general shape, these resemble the CO curves which were made during congestion. In each instance, the concentration of the dye in venous serum became constant within 12 to 15 minutes after injection. After release of the congesting cuffs, the concentration began to diminish.

3. *Total blood volume measured during rest and during venous congestion on different occasions*

Table I shows the results of measurements of the blood volume made under resting conditions and the volumes determined in the various congestion experiments described. In each case the concentration of CO or T-1824 found in the blood sample taken 20 minutes after delivery of CO or dye was used as the basis for calculations. As would be expected from inspection of the time-concentration curves, and from the results of the experiments in which CO-available volume was measured during congestion and just after release of the cuffs (Procedure 1), no significant differences were seen between the results of blood volume determinations made during massive congestion and those made under similar conditions without congestion.

4. *Measurements of the volume of blood contained in the congested extremities*

It was important to determine the approximate fraction of the total blood volume which had been contained in the congested extremities of each subject during the experiments described. Figure 4 illustrates the results of a typical experiment employing Procedure 4. The first column shows total CO-available volume and total erythrocyte volume while the congested extremities were excluded from the circulation by means of arterial tourniquets. Columns 2, 3, 4, and 5 show the changes in CO-available volume after release of the occluding and congesting cuffs. At the time of the final sampling, 11 minutes after release of the cuffs, an increase of 1370 ml. had occurred in CO-available volume, and of 610 ml. in total erythrocyte volume, amounting to increases of 25 per cent in both total volume and erythrocyte volume. Results of the three experiments carried out in this fashion are summarized in Table II. The increases of volume following release of arterial tourniquets were between 20 and 28 per cent of the final CO-available volumes; in other words, between one-fifth and one-fourth of the total blood volume of each subject was involved in the three congested limbs.

In each of the three experiments, release of the tourniquets and return of the previously pooled blood to more active circulation resulted in transient severe hyperpnea, a further evidence of the extent to which venous return from the limbs had

TABLE I

Summary of blood volume determinations made with CO and T-1824 under resting conditions and during congestion of three extremities by means of pneumatic cuffs

Subject	Date	Apparent total blood volume*		Cuff pressure	Indicator	Hematocrit
		Uncongested	Congested			
		ml.	*ml.*	*mm. Hg*		*ml./100 ml.*
J. J. S.	Feb. 5	5,360			CO	45.5
	Mar. 13	5,880			CO	45.5
	Apr. 1		5,880	60	CO	45.45
	Jul. 29		5,650	85	CO	48.3
	Apr. 17		5,830	60	T-1824	44.6
J. H.	Feb. 20	5,060			CO	46.55
	Mar. 27	6,100			CO	45.6
	Apr. 22	5,740			CO	45.55
	Apr. 3		5,640	70	CO	42.25
	Apr. 10		5,670	75	T-1824	43.35
E. B.	Dec. 3	4,800			CO	38.6
	Mar. 25	5,040			CO	39.3
	Apr. 8		4,710	70	CO	39.55
	Aug. 8		4,900	65	CO	40.8
	Apr. 15		4,590	64	T-1824	39.15

* Based on blood samples taken 20 minutes after delivery of the indicator.

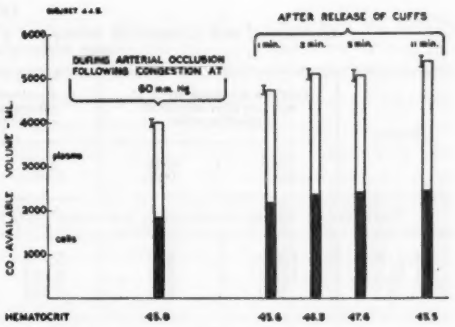

FIG. 4. CO-AVAILABLE VOLUME MEASURED DURING A SINGLE EXPERIMENT WHILE THE CIRCULATION TO BOTH LEGS AND ONE ARM WAS OCCLUDED BY CUFFS INFLATED TO 200 MM. HG, FOLLOWING 10 MINUTES OF CONGESTION AT CUFF PRESSURES OF 60 MM. HG (FIRST COLUMN) AND AT ONE, TWO, THREE AND 11 MINUTES AFTER RELEASE OF TOURNIQUETS (LAST FOUR COLUMNS)

Subject was connected to rebreathing system throughout. (Data are represented as in Figure 2.)

TABLE II

Increases of total CO-available volume and of erythrocyte volume, compared with final volumes after release of arterial tourniquets

Subject	Increase of volume 6½ to 11 minutes after release of occluding cuffs		Final volume 6½ to 11 minutes after release of occluding cuffs		Percentage increase after release of cuffs		Congesting pressure prior to occlusion (*mm. Hg*)
	Total (*ml.*)	Cells (*ml.*)	Total (*ml.*)	Cells (*ml.*)	Total (%)	Cells (%)	
Procedure 4—Limbs congested by pneumatic cuffs for 10 minutes before application of tourniquet pressure							
J. J. S.	1,370	610	5,370	2,440	25.5	25.0	60
J. H.	1,630	820	5,880	2,820	27.8	29.1	75
E. B.	920	420	4,680	1,870	19.7	22.5	65
Procedure 5—No congestion prior to application of tourniquet pressure							
J. J. S.	630	310	5,320	2,590	11.8	12.0	0
J. H.	900	440	5,740	2,700	15.7	16.3	0
E. B.	720	260	4,360	1,800	16.5	14.4	0

been impeded by the congesting cuffs. Similar hyperpnea did not follow release of the tourniquets in the experiments where arterial occlusion was not preceded by congestion (Procedure 5).

Comparison of the results obtained by Procedure 4 with those of Procedure 5, in which the volume of blood in the limbs was estimated without congestion, showed that, for each subject, the blood content of the extremities had been approximately doubled by congestion (see Table II).

DISCUSSION

The congestion which was imposed on the limbs in these experiments was made as extensive and as severe as possible, in order that circulatory stasis as it occurs clinically might be matched or exceeded. The experimental conditions could not simulate exactly the disturbances of blood distribution which may occur in heart failure, pregnancy, or shock for several reasons. First, the total blood volume of the healthy subjects was not increased, as it may be in congestive failure and pregnancy. Second, the experimental congestion was local and confined to the limbs rather than general and including the splanchnic and pulmonary circuits. Third, the pressure imposed on the veins of the experimental subjects was much higher than is ordinarily found clinically. Thus, whereas the total area of distribution of the congested regions might have been smaller, and the total volume of blood involved less, the intensity of the congesting pressure was considerably greater in the experimental situation than in any of the clinical states under consideration.

The results of experiments designed to measure the vascular volume of the limbs (Procedures 4 and 5) showed that a large fraction of the circulatory volume, in the neighborhood of one-fifth to one-fourth of the total blood volume, was contained in the congested regions under the conditions of the experiments. Further, it was shown that the vascular volume of the three extremities of each subject was approximately doubled by congestion. These estimates of volume, with and without congestion, are in surprisingly close agreement with results of similar studies made by others using slightly different techniques (11, 17). Use of a closed rebreathing system and of CO permitted measurement of blood volume while the tourniquets were in place and at intervals after release during a single experimental session. In the earlier studies in which the dye T-1824 was used, it was necessary to make determinations of blood volume, first with the circulation intact, and again, on another day, with arterial tourniquets in place (17).

It is probable that in all of these experiments involving arterial tourniquets, pressures distal to the occluding cuffs were slightly raised during the period of complete occlusion. For this reason, the grade of congestion which was present during measurement of vascular volume by means of Procedure 4 might have been somewhat higher

than that which existed during Procedures 1, 2, and 3, but the difference was presumably slight (18). It is certain that the rate of blood flow through the congested extremities was greatly diminished at the same time that the volume of blood contained in them was approximately doubled. Information is lacking on which to base an exact quantitative estimate of the decrement in flow (19).

It seems reasonable to conclude that the conditions of the experiments were sufficiently severe with respect to the volume of blood involved and the degree of obstruction to venous outflow, to permit critical testing of methods for measuring blood volume in clinical conditions characterized by massive pooling of blood.

The results of Procedures 1 and 2 have proved that the mixing period of 20 minutes which is usually employed with the CO method is more than sufficient for complete equilibration between the inspired gas and the entire volume of blood, even when a considerable portion of the circulating blood is moving at reduced speed in regions of congestion. Observations made with the dye T-1824 were somewhat less complete (Procedure 3, Table I), but these also support the conclusion that with sampling periods of 15 minutes or longer, and using either CO or dye, the total blood volume, and not merely the fraction of the blood which is circulating most rapidly, is reached by the indicator. These observations are in agreement with conclusions drawn from earlier experiments (20), in which the dye concentrations of venous bloods from upper and lower extremities were compared at various times after injection of dye during quiet standing and during recumbency.

At the time this series of experiments was being planned, it was thought that the time-concentration curves for CO and T-1824 which were made while the limbs were congested might prove to have distinctive contours as compared with curves made when congestion was not present (20–22). If this were the case, venous mixing curves might prove to have clinical value as a means of discovering the presence of vascular pools in regions where detection by more direct means is difficult, such as in the pelvic circulation during pregnancy. It was found, however, that the curves made with and without congestion were essentially the same in contour, even though in each pair of curves the one made during congestion had a slightly higher initial peak than the one made under resting conditions. Clearly, the presence of regional or general venous congestion could not be suspected, nor could any estimate concerning the relative magnitudes of "effective" versus total blood volume be made by simple inspection of individual mixing curves such as this if obtained under uncontrolled clinical conditions.

Conclusions are not justified here concerning the validity of plasma volume determinations made with T-1824 during limb congestion, because of the small number of experiments carried out with this substance. Difficulties have been encountered in using this method for studies of exercise (23) and shock where it became necessary to make indirect calculations of dye loss because of capillary leakage during the sampling period (24). When capillary pressure is greatly increased in the limbs, and probably to an even greater extent when visceral engorgement exists, the rates of loss of both fluid and protein from the blood stream are increased and the rate of lymphatic return uncertain (3, 25, 26). Under such circumstances, methods depending on labelling of erythrocytes have the advantage that total circulating red cell volume can be determined directly.

The results reported in this paper, together with a number of other observations (4, 20, 27–30) are in conflict with certain views which were expressed in the early literature on blood volume, concerning the nature of blood reservoirs (5). The brilliant studies of Barcroft on the functions of the spleen in animals (31) and certain studies by Wollheim on man (32a, b, c) provided a basis for the concept that important fractions of the blood, disproportionately rich in erythrocytes, could be sequestered in such depots as the spleen and the subpapillary venous plexus of the skin, where they might remain relatively static until returned to active circulation.

Barcroft's observations apparently do not apply to the spleen of normal man (4, 28–30). Wollheim was led by his experimental results to the conclusion that total blood volume, including both cell and plasma fractions, was diminished by several hundred milliliters during congestion which was similar in extent and intensity to that used in our experiments (32a, b, c). However, it can

be shown that the apparently conflicting results of the earlier studies were subject to a systematic error brought about by the use of premature sampling.

At the time Wollheim's studies were made, information was lacking concerning the time required for complete mixing of dyes in the blood stream. Inspection of the mixing curves obtained in the present study (Figure 3) demonstrates that at the sampling time used by Wollheim (three to six minutes), all of the curves were passing through their maxima, the peaks for the "congested" curves being higher than for the "uncongested" ones. Calculations based on samples taken at these time intervals after injection of the dye would lead to estimates of volume which are smaller than the true volume, and the error in this direction would be greater when congestion is present than under resting conditions.

It is possible to calculate, for each subject used in the present series, the apparent blood volume that would be obtained from the concentration of CO found in a five-minute and a 20-minute sample, with and without congestion, using the data from which Figure 3 was constructed. The data and results of these calculations appear in Table III and provide quantitative evidence concerning the magnitude of error which is introduced by premature sampling, both with congestion and without. The error in the direction of too small a CO-available volume is about twice as great (as much as 790 ml.) if the limbs are congested as that if there is no congestion (as much as 260 ml.). Examination of the time-concentration curves for T-1824 (Figure 3, right) shows that similar errors may be expected from premature sampling when this dye is used.

Although the results reported here are directly applicable only to the circulation in the extremities, they lend support to a relatively dynamic concept of blood volume reservoirs, in which these are regarded as regions of the circulation which are capable of accepting large volumes of blood, and through which blood of normal concentration continues to flow at varying rates. The relative accessibility of blood pooled in the limbs has been demonstrated by the marked similarities in shape between the time-concentration curves of CO made with and without congestion. Alterations in the dynamics of mixing were manifested only by the slight differences in contour appearing during the first 15 minutes after introduction of the gas. Thus, it is found that when sampling is delayed for at least 15 minutes after introduction of the indicator, *total erythrocyte volume*, whether measured with CO (27), as was done here, or indirectly by means of dyes (20), appears to be the same, with or without congestion. *Total blood volume* and *plasma volume* are smaller if measured during congestion only by the small fraction of plasma fluid which may be lost through filtration, while venous and capillary pressures are elevated during congestion (Figure 2) (20, 25–27).

It may be concluded that the presence of massive venous congestion does not cause delay in mixing of CO or of dyes to an extent which is sufficient to interfere with determinations of blood volume, provided blood samples are taken after an interval of not less than 15 minutes following

TABLE III

Comparisons between apparent CO-available volumes obtained from blood samples taken five minutes and 20 minutes after delivery of CO, under resting conditions and during congestion

Subject	Experiment	Volume of CO delivered	$C_f - C_i$		Calculated CO-available volume		Difference between result based on 5 min. and on 20 min. sample
			5 min.	20 min.	5 min.	20 min.	
		ml.	*ml./100 ml.*	*ml./100 ml.*	*ml.*	*ml.*	*ml.*
J. J. S.	Without congestion	113.1	2.05	1.92	5,520	5,880	−360
	With congestion	113.5	2.23	1.93	5,090	5,880	−790
J. H.	Without congestion	109.4	1.89	1.80	5,800	6,090	−290
	With congestion	111.4	2.25	1.97	4,950	5,650	−700
E. B.	Without congestion	113.9	2.26	2.26	5,030	5,030	0
	With congestion	115.4	2.66	2.45	4,350	4,710	−260

delivery of the indicator. Under such circumstances, total, rather than merely "effective," blood volume is measured.

SUMMARY

1. The blood volume of three healthy human subjects was measured by a CO method involving a closed rebreathing system (*a*) under resting conditions, and (*b*) during and after release of venous congestion of three extremities produced by pneumatic cuffs inflated to pressures just below the diastolic level.

2. Time-concentration curves for venous blood were made, using CO under resting conditions and CO and T-1824 during venous congestion.

3. The volume of blood contained in the congested extremities was measured and found to be in the neighborhood of one-fifth to one-fourth of total blood volume.

4. It was found that when a mixing time of 20 minutes or longer is allowed after delivery of the gas, CO-available volume is the same, whether or not venous congestion of the extremities is present.

5. Congestion of the extremities was found to produce consistent, slight changes in contour of time-concentration curves for CO, particularly during the earliest phases of mixing. These changes in contour were not sufficiently striking to permit use of such curves for estimating the presence or degree of venous pooling. Because of the characteristics of the curves, the use of sampling periods shorter than 15 minutes may cause errors in the direction of falsely low blood volumes.

6. It is concluded that the presence of massive congestion of the extremities does not interfere with complete admixture of CO or T-1824 with the entire blood volume within 20 minutes, and that with the CO method employed, total, rather than only "effective," blood volume is measured.

7. The results have been discussed in relation to some concepts concerning the nature of blood volume reservoirs.

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QUANTITATIVE ANTIBODY STUDIES IN MAN. I. THE EFFECT OF ADRENAL INSUFFICIENCY AND OF CORTISONE ON THE LEVEL OF CIRCULATING ANTIBODIES[1]

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Investigators have for many years been interested in the relation of the adrenal gland to antibody formation. Observations made in animals have frequently led to conflicting results. The effect of adrenalectomy on antibody titres has been studied and has been shown to be associated with an elevation (1–3), a depression (4), and no change (5–8). The effect of ACTH or cortisone on antibody levels in the acute experiment has resulted in an elevation (1, 5, 9–11), no change (8, 12), and a depression (13). The effect of ACTH or cortisone on antibody levels in the chronic experiment has resulted in an elevation (9), a depression (14, 15) and no change (16, 17). Observations in man on the effects of ACTH or cortisone on antibody levels have failed to demonstrate a change in either the acute (18–20) or chronic (21, 22) experiment. In previous studies on humans, antibody measurements have not been based on techniques which lend themselves to precise measurement. The purpose of this paper is to present observations on the effect of adrenal insufficiency and of cortisone administration on antibody titres in man using quantitative immunochemical techniques.

MATERIALS AND METHODS

The method of Heidelberger and MacPherson for the quantitative determination of precipitable antibody nitrogen was used throughout the experiments (23). Type-specific capsular polysaccharides of pneumococcus types I and II were used as antigens. Since complement and C-antibody may add nitrogen to specific precipitates (24, 25), these were removed before the analysis of the specific precipitates was carried out. Since heat inactivation may damage antibody present in low concentration (25), all sera were de-complemented with a system of crystalline ovalbumin and rabbit antiovalbumin serum. The C-antibody was removed with C-polysaccharide derived from a type III or type VII pneumococcus. Results are expressed as micrograms of precipitable antibody nitrogen per 4 milliliters of serum. All materials were supplied through the generosity of Dr. Michael Heidelberger.

Individuals used in this study were from the hospital and clinic population of the Presbyterian Hospital. The patients used as controls were diagnosed as having peptic ulcer or arteriosclerotic heart disease. The five patients with adrenal insufficiency had the characteristic clinical and laboratory findings of Addison's disease. Desoxycorticosterone acetate was administered to four of the five patients during the test period. Although there is little evidence available in the literature, it has been shown that in animals DCA does not appear to influence antibody titres (10). The cortisone treated group were patients with rheumatoid arthritis receiving 200 milligrams of the drug by mouth three times each week. Cortisone treatment was started at least two weeks before immunization and was continued throughout the test period. No other medications were given. As an additional control group, eight patients with untreated rheumatoid arthritis were included in this study.[3] Following a preliminary bleeding, each of the subjects was given 0.08 milligrams each of types I and II pneumococcus polysaccharides subcutaneously. Post-immunization bleedings were obtained at two and four weeks.

RESULTS

As can be seen from Table I, the 12 normal individuals exhibited responses similar to those observed by Heidelberger and associates (25, 26). C-antibody was present in all sera tested and there was no rise in the titres of C-antibody in spite of sizeable changes in antibody to the type-specific capsular polysaccharides. Pre-immunization antipolysaccharide titres were low or not detectable. Maximum antibody titres were usually achieved by two weeks after immunization. A good response to one antigen was not necessarily accompanied by a similar response to the other antigen.

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[2] John and Mary R. Markle Scholar in Medical Science.

[3] Patients with arthritis used in this study were made available through the courtesy of Dr. Charles Ragan.

TABLE I

Antibody formation in normal humans

Subject	Antibody to*	Micrograms of antibody nitrogen per 4 milliliters of serum	
		Pre-immunization	2 weeks post-immunization
1	C III	46	28
	S I	3	11
	S II	0	11
2	C III	21	18
	S I	1	27
	S II	1	23
3	C III	50	57
	S I	4	30
	S II	1	23
4	C III	7	6
	S I	0	29
	S II	1	45
6	C III	32	25
	S I	0	0
	S II	0	6
9	C III	18	17
	S I	1	4
	S II	0	7
11	C III	60	62
	S I	1	15
	S II	8	24
35	C III	7	8
	S I	0	52
	S II	0	96
39	C III	26	26
	S I	0	15
	S II	0	22
40	C III	39	50
	S I	1	144
	S II	3	61
41	C III	12	8
	S I	0	9
	S II	0	19
52	C III	33	48
	S I	0	22
	S II	2	15

* Letter and number refer to specific polysaccharide precipitinogen used.

TABLE II

Antibody formation in patients with adrenal insufficiency

Subject	Antibody to*	Micrograms of antibody nitrogen per 4 milliliters of serum	
		Pre-immunization	2 weeks post-immunization
28	C III	10	7
	S I	0	25
	S II	1	101
29	C III	23	32
	S I	0	30
	S II	0	39
32	C III	20	23
	S I	0	7
	S II	0	8
37	C III	14	9
	S I	0	15
	S II	0	26
33	C III	11	5
	S I	1	20
	S II	2	22

* Letter and number refer to the polysaccharide precipitinogen used.

Table II shows the data obtained on the antibody formation in five patients with adrenal insufficiency. It can be seen that there is no departure from the behavior of the control group.

In Table III data are presented on eight patients with untreated rheumatoid arthritis. It is apparent that the responses are similar to those observed in the group of controls. Table IV includes the figures for nine patients in the cortisone-treated group. There does not appear to be any impairment of antibody formation among those receiving cortisone, and, indeed, three of the nine patients demonstrated unusually high post-immunization titres.

Data are presented in Table V on two patients with rheumatoid arthritis who were immunized two weeks before the administration of cortisone. Patient No. 64 received 300 milligrams of cortisone and patient No. 67 received 200 milligrams of cortisone on the first day of treatment. Both patients were then given 100 milligrams of cortisone daily over the next four weeks. Antibody titres were determined at three hour intervals in the period immediately following the first dosage of cortisone as well as after four weeks of the drug. The data indicate that with the dosages used, cortisone exhibited no effect on previously elevated antibody titres.

COMMENT

Some of the conflicting evidence in the literature on the relation of adrenal activity to antibody levels may be resolved on the basis of species differences, as suggested by Mirick (21). Many of the results were obtained with techniques which do not permit direct comparison of the data from one

TABLE III

Antibody formation in patients with untreated rheumatoid arthritis

Subject	Antibody to*	Micrograms of antibody nitrogen per 4 milliliters of serum		
		Pre-immunization	Post-immunization	
			Two weeks	Four weeks
64	C VII	26	49	†
	S I	0	46	†
	S II	0	56	†
67	C VII	38	36	†
	S I	2	2	†
	S II	2	50	†
98	C VII	2	4	6
	S I	0	7	7
	S II	1	17	20
99	C VII	10	10	10
	S I	0	22	26
	S II	0	10	12
100	C VII	34	39	44
	S I	4	93	146
	S II	2	13	14
101	C VII	97	108	92
	S I	0	3	3
	S II	0	0	2
102	C VII	29	35	31
	S I	1	76	112
	S II	3	23	20
103	C VII	13	12	12
	S I	0	18	13
	S II	0	15	17

* Letter and number refer to the polysaccharide precipitinogen used.
† See Table V.

TABLE IV

Antibody formation in cortisone-treated patients with rheumatoid arthritis

Subject	Antibody to*	Micrograms of antibody nitrogen per 4 milliliters of serum		
		Pre-immunization	Post-immunization	
			Two weeks	Four weeks
77	C VII	9	16	32
	S I	1	15	20
	S II	0	120	72
78	C VII	25	38	34
	S I	0	67	40
	S II	0	80	65
79	C VII	48	41	43
	S I	0	21	27
	S II	3	22	18
87	C VII	7	4	42
	S I	0	0	0
	S II	0	12	18
88	C VII	39	61	61
	S I	0	126	113
	S II	0	110	170
89	C VII	14	60	10
	S I	0	0	0
	S II	0	28	35
90	C VII	31	53	40
	S I	0	44	38
	S II	19	36	31
91	C VII	14	20	21
	S I	0	89	106
	S II	4	104	156
92	C VII	22	26	27
	S I	3	155	196
	S II	2	116	90

* Letter and number refer to polysaccharide precipitinogen used.

laboratory to another. Three groups of observers, however, using precise immunochemical techniques, have described the effect of cortisone and ACTH on antibody formation in the rabbit. DeVries reported the effect of a single dosage of 50 milligrams of ACTH on the titre of rabbit anti-egg albumin in an acute experiment (13). Some of the animals were noted to have a significant drop in precipitable antibody nitrogen within nine hours, but as noted by the author, a serum dilution effect may have influenced the results. Germuth and Ottinger noted a striking decrease in rabbit anti-egg albumin production when cortisone in dosage of 10 milligrams per day was administered concomitantly with the antigen (15). ACTH in dosage of 10 milligrams per day produced a similar but less marked effect. Bjørneboe, Fischel, and Stoerk studied the production of polyvalent rabbit anti-pneumococcal serum using 10 milligrams per day of cortisone and up to 3 milligrams per day of ACTH (14). Their results were in agreement with those obtained by Germuth, although somewhat less dramatic. In addition, the effect was the same whether the drugs were given during the period of active immunization or to animals with previously elevated antibody titres. The dosages of cortisone used in the experiments on rabbits were considerably in excess of those used clinically when calculated on the basis of body weight.

There is evidence to suggest that the amount of antibody produced may be in part dependent upon

TABLE V

Effect of cortisone on previously elevated antibody titers in two patients with rheumatoid arthritis

Subject	Antigen to‡	Micrograms of antibody nitrogen per 4 milliliters of serum						
		Pre-immunization	2 weeks post-immunization	Time after cortisone				
				3 hrs.	6 hrs.	9 hrs.	12 hrs.	4 wks.
64*	C VII	26	49	49	48	48	31	35
	S I	0	46	48	45	43	43	37
	S II	0	56	63	59	56	50	52
67†	C VII	38	36	43	31	35	34	20
	S I	2	2	2	1	2	3	6
	S II	0	50	57	54	56	52	44

* Initial dosage cortisone 300 mg.
† Initial dosage cortisone 200 mg.
‡ Letter and number refer to polysaccharide precipitinogen used.

a local inflammatory reaction at the site of deposition of the antigen and cortisone in adequate dosage may inhibit this inflammatory reaction and thus interfere with a maximal antibody response (27, 28). During the course of these experiments, no clinical difference was observed in the local inflammatory response to the antigen in the cortisone-treated group as opposed to the control group. Moreover, there did not appear to be any relation between the severity of the local reaction and the subsequent antibody titre. The failure of cortisone to influence circulating antibody titres in man may be a reflection of dosage as well as a species difference.

The conflicting evidence on antibody formation in adrenalectomized animals is difficult to explain. Early studies (2–4, 6) were done on adrenalectomized animals not maintained on any specific replacement therapy and the protocols indicate a high post-operative mortality rate. Jaffe and Marine (2), however, noted that the increased antibody formation was apparent only if the antigen was given soon after adrenalectomy. With a six-week interval between adrenalectomy and immunization, there was no increase in antibody formation. The present studies are not strictly comparable to those carried out in adrenalectomized animals since patients with Addison's disease may conceivably have some functional adrenal cortical tissue. The finding that patients with adrenal insufficiency do not exhibit any impairment or increase in antibody formation is in agreement with the quantitative data obtained in animals by Eisen and associates (5) and hemolysin formation as reported by Thatcher, Houghton, and Ziegler (8).

SUMMARY

1. Quantitative immunochemical studies have been carried out in man using pneumococcal capsular polysaccharides as antigens.

2. The 12 subjects used as controls showed the presence of C-antibody in all sera tested. The C-antibody remained relatively constant in spite of sizeable changes in antibody to the capsular polysaccharides. There is considerable variation in the antibody response from one individual to another. A large response to one antigen is not necessarily accompanied by a similar response to another antigen in the same individual. Maximum antibody titres are usually achieved by two weeks following immunization.

3. The five patients with adrenal insufficiency did not demonstrate any impairment of ability to form antibodies.

4. The eight patients with untreated rheumatoid arthritis and nine cortisone-treated patients with rheumatoid arthritis had similar antibody responses when observed over a period of four weeks.

5. Two patients with rheumatoid arthritis with previously elevated antibody titres showed no change in these titres on the acute or chronic administration of cortisone.

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THE RENAL RESPONSE TO INTRAVENOUS INJECTION OF SODIUM CHLORIDE SOLUTIONS IN MAN [1]

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The administration of isotonic saline solution to dogs in quantities equal to one-half or more of the volume of extracellular fluid leads to a prompt increase in glomerular filtration rate and the excretion of sodium and chloride. Although factors possibly influencing tubular reabsorptive activity, such as the adrenal and neurohypophysial hormones, are not as yet amenable to quantitative study, the general correlation between filtration rate and sodium excretion in the dog has led many investigators to believe that changes in glomerular activity constitute one of the most important mechanisms in the maintenance of salt and water balance in this animal (1–5). So effective is this renal response that, with other adjunctival mechanisms, dogs may be maintained on diets containing 4 gm. of salt per kg. per day (corresponding to 280 gm. per day in a 70 kg. man) without gain in weight or apparent disturbance in salt and water balance (5).

An extensive literature is available (6) on the excretion of sodium, chloride and water in patients receiving saline infusions, but in great part the information contained therein is merely descriptive of the fact of diuresis (or absence of diuresis) and is not correlated with the measurable physiological mechanisms concerned in the excretion of sodium and water. No information is available on the effect of saline solutions on renal function in man, and the present study was undertaken to obtain information, comparable to that available on the dog, on the changes in filtration rate, renal blood flow and electrolyte excretion after the rapid administration of saline infusions.

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METHODS

Our observations were made on 35 ambulatory patients, ranging in age from 19 to 75 years, who had been hospitalized for relatively benign conditions, who had not undergone surgery, and who were ready for discharge. None presented clinical evidence of cardiac or renal disease. All were afebrile and maintained on a regular ward diet.

The patients drank a liter of water the night before observation. This water was consumed in divided doses at the patients' convenience. After a light breakfast, they were brought to the research unit for renal function studies at about 8:00 a.m. After appropriate priming injections, a constant infusion of sodium p-aminohippurate (PAH) and inulin (IN) in isotonic saline solution was given at the rate of 0.6 cc./min. during the entire observation period through a flexible intravenous catheter by means of a constant infusion pump. After three or more control urine-collection periods of approximately 30 minutes each, 1 to 3 liters of isotonic saline solution were administered intravenously at a rate of 13 to 56 cc./min. The administration of PAH and inulin was maintained throughout the saline infusion and for approximately 210 minutes thereafter. Urine was collected every 30 minutes by sterile urethral catheterization and the bladder rinsed with sterile distilled water. Blood samples were drawn from a peripheral vein at hourly intervals; heparin was added as an anticoagulant.

Inulin was determined by Harrison's method (7), PAH by the method described by Smith and his associates (8), chloride by the method of Van Slyke and Hiller (9), uric acid by the Benedict and Franke method (10), creatinine by the Bonsnes-Taussky method (11), sodium and potassium by a Perkin-Elmer internal standard flame photometer, plasma proteins by the copper sulfate method (12) and the hematocrit by centrifuging heparinized blood in a Wintrobe hematocrit tube for 20 minutes.

The effects of saline infusion on renal clearances were followed in 21 subjects. In addition, the protracted effects of saline infusion on urine flow and electrolyte excretion were examined in eight additional subjects without observation on renal clearances; in these subjects, the urethral catheter was removed about 200 minutes after the end of the saline infusion, and urine was collected by spontaneous voiding for 16 to 28 hours. Four subjects were given 9 to 24 gm. of sodium chloride daily, either orally or intravenously in the form of isotonic saline solu-

tion, in addition to their dietary intake during a period of 48 to 96 hours previous to the test. Six subjects received approximately 500 cc. of a 5 per cent solution of sodium chloride at a fairly rapid rate (24 to 39 cc./min.) and were observed for 210 minutes after the infusion had ended.

Clearance measurements were not started until three hours after breakfast and no food or water was given throughout the period of clearance determination. The patients in whom urine excretion was followed overnight received a light supper and breakfast, with omission of salt. All fluid intake was recorded.

Inulin and PAH clearances and urine concentrations and electrolyte excretion rates have been integrated by multiplying the observed rate in each urine collection period by the elapsed time, in order to determine the mean rate per minute. Water and electrolyte recovery in the urine has been calculated as the per cent of the quantity administered, after correction for basal excretory rate. The latter has been taken as the integrated average excretion during the three control periods, except in the case of those subjects whose control urine flow exceeded 1.5 cc./min.; such subjects were usually on the descending limb of water diuresis and, on the assumption that basal urine flow could be expected to decrease to or below 1.5 cc./min., this figure is arbitrarily used for this correction. No method of correction for spontaneous urine output is wholly reliable and this circumstance introduces some error into all long period calculations of the return of both water and electrolytes. No correction is made for insensible water loss. In some cases the bladder was emptied immediately at the end of the saline infusion and the urine discarded; the estimated recovery of fluid in these instances is consequently somewhat lower than the true value.

Changes in hematocrit, plasma electrolyte and protein concentration are expressed as the greatest percentile change observed during the post-infusion period, relative to control values.

RESULTS

Istonic Saline Solution

A summary of pertinent data is given in Table I, which records averages of control data integrated over three control periods as noted above, and the average changes induced by isotonic saline solution as revealed by integrated post-infusion rates.

Urine flow, inulin, and PAH clearances. The intravenous administration of 1 to 3 liters of isotonic saline solution increased the urine flow above control rates in 90 per cent of 29 subjects examined. Except in a few instances, however, the diuresis was much less than would be expected from the same quantity of water ingested orally. This difference between saline and water diuresis in man has long been recognized (13) but it is emphasized in few recent publications. The maximal rate of urine flow in our subjects usually occurred during the latter part of the infusion period or in the first 30 minutes thereafter and was followed by a steady decline toward the control value. There was no apparent relation between the quantity of fluid given or the rate of administration and the extent of diuresis.

The saline solution had no consistent effect upon the inulin clearance in 21 subjects maintained on a ward diet, as shown by the average figures in Table I, or in four additional subjects who had been on a high salt intake previous to the saline infusion. The PAH clearance was no more markedly affected in 20 subjects. The direction of change in the two clearances coincided in only one-fourth of the observations. In one subject the PAH clearance increased by 103 per cent, but it had been anomalously low in the control periods, yielding a filtration fraction of 0.38; here the inulin clearance remained unchanged after infusion, the filtration fraction decreasing to 0.19. In the four subjects who had been maintained on a high salt intake, 3 liters of isotonic saline solution caused an average increase in PAH clearance of 13 per cent without appreciably changing the inulin clearance. In neither group could changes in the inulin or PAH clearances be correlated with changes in urine flow, with the rate or quantity of fluid administered, or with the rate of sodium or chloride excretion in the control periods.

Sodium. The most consistent effect of the administration of isotonic saline in the present studies was the increase in the excretion of sodium, which was followed in 15 subjects on the ward diet. This increased excretion was the result of an increase in concentration in the urine as well as in the rate of urine flow. Average urine sodium concentration during the 210 minute post-infusion period increased in 12 subjects, the increase averaging 25 per cent. In one subject this figure decreased while it remained unchanged in two.

Control sodium excretion on the ward diet in these subjects averaged 0.15 mEq./min. (0.075) or 13 gm. of sodium chloride per day. This figure invariably increased after isotonic saline infusion. Except in three instances the increment in sodium excretion exceeded that in chloride excretion, but the greatest difference between sodium and chlo-

ride in favor of the former was 0.066 mEq./min. In the four subjects on a high salt diet the control urine sodium concentration averaged 180 mEq./L. In three of these this figure increased after infusion (36, 94, and 229 per cent), while in the fourth it decreased by 50 per cent (from 368 to 184 mEq./L.). Control sodium excretion in these subjects averaged 0.326 mEq./min., equivalent to 27.4 gm. of sodium chloride per day. Sodium excretion increased after infusion in all four subjects; the average increase was equivalent to 43 gm. of sodium chloride per day.

Chloride. After saline infusion the urine chloride concentration increased in 12 and decreased in nine subjects on the ward diet, but in only one instance was the increase marked (450 per cent); the average positive change, excluding this subject, was only 14 per cent. The four subjects on a high salt intake showed an average increase of 51 per cent.

Control chloride excretion on the ward diet averaged 0.19 mEq./min., or 15.7 gm. of sodium chloride per day. This high figure probably reflects both a high dietary intake and the effects of the sodium chloride administered with the inulin and PAH. Chloride excretion increased after saline infusion in 17 subjects; the increase averaged 100 per cent; in the other four subjects it decreased by an average of 14.3 per cent, to give an average for the entire group of + 59 per

TABLE I

Changes in renal function induced by the intravenous administration of isotonic saline solution to patients maintained on a regular ward diet

Subjects	V		Hematocrit		Protein		C_{IN}		C_{PAH}		U_{Cl}	
	Control	Maximal post-infusion	Control	Lowest post-infusion	Control	Lowest post-infusion	Control	Post-infusion	Control	Post-infusion	Control	Post-infusion
	cc./min.		*per cent*		*grams*		*cc./min.*		*cc./min.*		*mEq./L.*	
A. T.	7.4	4.4	50.5	44.0			151.7	131.0	536	644	32	177
M. G.	1.8	20.0	46.0	40.2			115.7	103.0	508	551	131	200
E. J.	1.3	4.3	47.6	45.1			81.3	111.3	682	467	126	96
C. P.	3.2	1.5	45.3	45.7			104.2	101.4	661	719	93	181
M. R.	3.4	15.2	43.9	46.6	5.90	5.60	160.5	185.2	586	610	51	27
J. P.	1.7	2.1	50.7	38.1	5.06	4.65	107.1	99.0	779	826	132	209
J. F.	1.4	5.2	45.2	38.6	5.15	3.99	115.3	83.3	586	751		
V. H.	1.1	4.6	37.3	36.3	5.55	3.90	126.2	125.5	307	624		
T. A.	2.8	17.6	45.2	40.4	5.00	3.52	109.0	122.0	833	847		
T. A.	1.4	10.2	38.7	35.2	5.75	4.25	110.0	117.2	736	689		
T. S.	2.2	8.1	44.6	39.1	5.35	4.27	119.0	122.7	581	577		
T. S.	2.0	7.3	38.2	34.2	5.00	4.10	115.3	122.2	603	637		
M. B.	4.3	3.7	42.9	36.8	5.93	5.00	139.8	139.8	609	623		
G. J.	3.5	5.6	35.4	30.7	5.36	3.91	154.0	157.6	655	596		
J. L.	1.0	4.5	45.5	40.0	4.64	3.90	118.9	126.3	572	667	196	217
J. T.	0.5	1.2	50.5	46.5	7.22	5.36	202.0	149.0	604	702	247	241
T. F.	1.3	2.6	46.5	41.2	5.35	4.28	114.2	109.8	525	524	157	144
J. S.	3.2	6.2	45.2	42.8	5.00	4.25	122.8	124.4	554	542	82	149
J. S.	1.2	2.0	51.0	39.6	5.35	4.49	100.3	105.0	483	456	92	108
O. B.	1.3	4.8	43.4	36.5	6.50	5.55	59.6	71.9	356	398	96	123
S. N.	1.7	2.1	48.8	45.9	5.75	5.35	204.0	164.0	1060	905	229	238
J. O.	1.0	3.6	41.0	40.7	4.82	4.65					175	159
J. M.	1.1	3.2	49.8	45.3	5.35	4.65					154	147
J. S.	1.0	4.1	41.8	36.4	4.65	3.90					105	100
E. C.	0.4	3.1	41.3	39.6	5.00	4.80					123	186
M. J.	1.1	4.8	36.7	32.6	5.00	4.10					149	78
W. G.	0.8	1.2	38.5	35.2	5.20	4.65					174	220
J. M.	2.1	12.3	41.5	39.2	4.80	4.27					125	135
J. J.	0.8	4.2	41.8	38.5	5.00	4.28					224	177
Average	1.9	5.2	43.9	39.7	5.35	4.47	125	124	610	636	138	157
Standard deviation		3.9		3.1		0.48		20		107		51
Probability		.001 Signif.		.001 Signif.		.001 Signif.		.6 Not signif.		.3 Not signif.		.1-.05 Not signif.

TABLE 1—*Continued*

Changes in renal function induced by the intravenous administration of isotonic saline solution to patients maintained on a regular ward diet

Subjects	$U_{Cl}V$		U_{Na}		$U_{Na}V$		U_K		U_KV		Recovery in 210 minutes		
	Control	Post-infusion	Control	Post-infusion	Control	Post-infusion	Control	Post-infusion	Control	Post-infusion	H_2O	Cl	Na
	mEq./min.		*mEq./L.*		*mEq./min.*		*mEq./L.*		*mEq./min.*			*per cent*	
A. T.	.237	.500									*18.0	*21.8	
M. G.	.230	.226									*−3.7	*−0.3	
E. J.	.163	.258									*22.6	*8.7	
C. P.	.297	.255									*−1.4	*−6.5	
M. R.	.162	.115									70.8	*−6.0	
J. P.	.222	.493									9.3	25.4	
J. F.											38.7		
V. H.											*6.9		
T. A.											47.3		
T. A.											136.5		
T. S.											23.2		
T. S.											24.4		
M. B.											9.2		
G. J.											20.5		
J. L.	.198	.469	163	203	.165	.434	92	38	.095	.074	9.7	40.2	40.0
J. T.	.114	.201	194	204	.090	.171	101	82	.046	.068	9.7	15.6	14.5
T. F.	.188	.164	108	128	.129	.150	108	96	.129	.110	0	−3.6	3.1
J. S.	.237	.411	77	145	.217	.400	18	18	.052	.051	35.7	27.7	28.8
J. S.	.109	.163	107	124	.126	.186	52	43	.061	.065	9.5	8.1	8.9
O. B.	.120	.337	131	143	.165	.392	46	19	.057	.050	36.0	33.5	35.1
S. N.	.390	.394	185	214	.222	.360	96	63	.162	.101	3.4	0.6	29.6
J. O.	.192	.299	177	172	.204	.322	60	27	.070	.051	22.0	14.0	15.3
J. M.	.166	.266	165	157	.179	.280	67	25	.072	.046	19.8	15.6	15.8
J. S.	.101	.230	85	105	.083	.237	72	33	.069	.071	38.7	20.9	25.0
E. C.	.054	.238	171	234	.075	.286	44	26	.020	.030	19.7	32.3	37.0
M. J.	.150	.201	155	80	.155	.203	51	22	.050	.057	31.3	6.4	4.9
W. G.	.134	.188	173	235	.132	.200	91	63	.069	.049	1.6	9.9	12.3
J. M.	.256	.358	129	143	.263	.424	24	15	.049	.035	35.2	14.9	23.6
J. J.	.172	.406	138	151	.107	.353	156	53	.106	.102	0.6	11.0	14.1
Average	.185	.294	144	163	.154	.293	72	41	.074	.064	24.0	14.0	17.0
Standard deviation		33		83		77		25		19			
Probability		.001 Signif.		.1–.05 Not signif.		.001 Signif.		.001 Signif.		.1–.05 Not signif.			

* Washout period during saline infusion not included.

cent. Chloride excretion in the four subjects on a high salt intake averaged 0.338 mEq./min. and increased after isotonic saline infusion by an average of 274 per cent.

Potassium. The urine potassium concentration in 15 subjects on a ward diet decreased after isotonic saline infusion in all but one subject, who showed no change. Potassium excretion was randomly affected, increasing in five instances by 3 to 50 per cent and decreasing in nine (− 4 to − 37 per cent). Urine potassium concentration likewise decreased after infusion in the four subjects on a high salt diet (average − 30 per cent) but the rate of excretion changed significantly in only one (from 0.028 to 0.122 mEq./min.).

Plasma changes. Plasma and sodium chloride concentrations were changed minimally by the infusion of 1 to 3 liters of isotonic saline solution. Potassium concentration was more markedly affected, but the changes were random in direction and ranged from − 10 to + 32 per cent of control levels. In the four patients on a high sodium chloride intake the saline infusion increased the plasma chloride concentration by + 5 to + 13 per cent and the plasma sodium by 0 to + 4 per cent, while the plasma potassium concentration changed by − 4 to + 19 per cent.

The hematocrit decreased in the majority of patients immediately after the administration of isotonic saline solution, returning toward but not

to initial levels at the end of the observation period. The average maximal decrease was only 8 per cent. The concentration of plasma protein also decreased in all cases immediately after saline infusion, with a gradual return toward normal; the average maximal decrease was 16 per cent.

The progressive return of both hematocrit and plasma protein concentration toward control levels cannot represent solely the distribution of saline between the circulatory tree and the interstitial fluid. Isotope studies indicate that 60 per cent of plasma sodium and chloride and 140 per cent of the plasma water are exchanged with the extravascular compartment per minute in the guinea pig. In man, 78 per cent of the plasma sodium and 105 per cent of the plasma water are exchanged with extravascular sodium and water per minute (14–16). Assuming that the extracellular fluid represents 16 per cent of the body weight (17, 18), 3 liters of saline should dilute the 11 liters of extracellular fluid in a 70 kg. man by 21 per cent. In the light of the data cited above one would expect rapid and persistent dilution of the hematocrit and plasma protein to this extent. The facts that the maximal decrease in both values is much less and that both return toward normal before the saline is excreted (*vide infra*) indicate that a substantial fraction of the saline escapes from the extracellular into the intracellular compartment.

Recovery of salt and water. In the 21 subjects on the ward diet, the recovery of administered water as urine (with correction for basal flow, as noted under Methods) at the end of 210 minutes after termination of the infusion averaged only 24 per cent. In only one subject did water recovery exceed 100 per cent, while in nine recovery was less than 10 per cent. Recovery of chloride in 21 subjects averaged 14 per cent and recovery of sodium 18 per cent.

In eight subjects urine was collected for 16 to 28 hours after isotonic saline infusion, in anticipation of a possible delayed excretion of water or electrolyte. (We recognize that such recoveries, corrected as they must be for the basal excretion of urine and electrolyte, are subject to large error because of the diurnal variation in water and electrolyte excretion and other uncontrolled factors.) The calculated recovery of water, sodium and chloride decreased progressively the longer the study was carried on in four subjects, while in the other four subjects recoveries appeared to remain at fairly constant levels or to increase slightly the longer the subject was observed. However, at no time did the recovery approach the quantity of fluid or electrolyte administered.

The administration of 9 to 24 gm. of sodium chloride per day for a week previous to the experimental procedure had no effect on the immediate recovery of either water or electrolytes; the recovery of water averaged 18 per cent, of chloride 21 per cent, and of sodium 16 per cent.

Hypertonic Sodium Chloride Solution

Inulin and PAH clearances. In the six subjects who received 500 cc. of 5 per cent sodium chloride solution the urine flow increased above the control level in all and reached peak levels of 2.4 to 7.3 cc./min. The filtration rate increased in three (16, 19, and 98 per cent) and decreased in three (2, 6, and 7 per cent). The PAH clearance increased in five (11 to 90 per cent) and decreased in one (7 per cent).

Chloride. The urine chloride concentration increased in all, and reached values in the range of 232 to 265 mEq./L. Chloride excretion increased to the range of 0.46 to 1.58 mEq./min.

Sodium. Urine sodium concentration increased in all six subjects to levels of 216 to 265 mEq./L., while sodium excretion reached levels of 0.49 to 1.41 mEq./min. Urine concentrations of both sodium and chloride after the administration of hypertonic saline solution were always well above the plasma concentrations. Urine potassium concentration increased in some instances and decreased in others, the average of all cases being + 30 per cent, while the excretion of potassium increased by an average of + 82 per cent.

Since the amount of fluid administered was small (500 cc.) no attempt was made to estimate water recovery, but the increase in urine flow was insufficient to effect substantial recovery of water. Sodium recovery in approximately 210 minutes averaged 24 per cent and chloride recovery averaged 25 per cent, figures only moderately greater than were obtained with isotonic saline solution.

The average maximal decrease in the hematocrit was 15 per cent, in plasma protein concentration, 13 per cent, figures which are again disproportionately low considering the quantity of sodium

chloride administered, a quantity which might be expected to effect marked hemodilution by expansion of the extracellular fluid at the expense of intracellular water.

Changes in the electrolyte composition of the plasma were, of course, more marked after hypertonic than after isotonic saline solution. The plasma chloride concentration increased by 10 to 20 per cent and the concentration of sodium by 7 to 10 per cent, while the changes in the plasma potassium concentration ranged from − 19 to + 36 per cent.

Uric Acid/Creatinine Excretion Ratio

In view of the possibility that adrenal cortical activity might be altered by the administration of saline, and that a change in cortical activity might be reflected in the uric acid/creatinine excretion ratio, this ratio was followed in eight subjects before and after the administration of isotonic saline, and in five subjects before and after the administration of 5 per cent saline. The changes in the ratio were, however, so slight that we do not consider them to be significant.

DISCUSSION

In contrast to the renal response observed in the dog, the intravenous administration of isotonic saline solution in quantities up to one-quarter of the extracellular fluid volume does not consistently induce a sustained increase in filtration rate or renal plasma flow in human subjects. In 11 of 21 subjects tested, the filtration rate after the infusion ended either decreased or underwent less than a 5 per cent increase. Despite the absence of a consistent increase in filtration rate, the excretion of sodium and chloride was substantially increased in nearly all subjects. There was no correlation between the changes in filtration rate and in the excretion of these electrolytes. Since the plasma concentration of sodium was not significantly altered, it must be concluded that the intravenous administration of isotonic and hypertonic saline solutions generally leads to increased sodium chloride excretion in consequence of diminished tubular reabsorption at a constant filtered load.

The effects of isotonic saline solution upon the excretion of water are difficult to evaluate. In three instances the administration of this solution led to the excretion of a urine substantially more dilute in sodium and chloride than the plasma. This anomalous response simulated the water diuresis shown by subjects who have been prehydrated with 1500 to 2000 cc. of water eight to 13 hours previous to the administration of saline, as reported by Ladd (19, 20) since this study was completed. On the whole, however, our subjects did not show the copious excretion of either sodium or water which Ladd reports. We are inclined to agree that the important difference between his subjects and ours lies in the quantity of water consumed the evening before the saline test, and perhaps in the speed with which this water is ingested.

The administration of 5 per cent sodium chloride solution also led to increased sodium and chloride excretion regardless of whether the filtration rate increased or not, though the recovery of water and electrolyte was not materially greater than with isotonic saline. In no instance did the urine sodium or chloride concentration fall below that of plasma in the post-infusion phase.

Wesson and his associates (3) have shown in the dog that the tubular reabsorption of sodium at a constant filtered load is increased when the extracellular fluid volume is expanded without change in electrolyte composition. Our data clearly demonstrate that in man the rapid administration of saline (though without effort to sustain the expansion of the extracellular fluid volume) has the opposite effect: namely, to reduce tubular reabsorption at essentially constant filtered loads. The conditions of our experiments are not identical with those of Wesson and his co-workers, but they are sufficiently similar to suggest that man and the dog differ not only in respect to the effects of expansion of the extracellular fluid upon the filtration rate, but also in respect to changes in tubular reabsorption at a constant filtered load. Tubular reabsorption is increased in the dog but decreased in man under these roughly parallel conditions. It seems probable, nevertheless, that the absence of an increase in filtration rate accounts for the fact that man excretes sodium chloride, no matter how administered, so much more sluggishly than does the dog.

The similarity of the response in subjects who had been maintained for several days on a high salt diet with that obtained in patients maintained on a ward diet indicates that neither the failure to obtain an increase in glomerular filtration nor the

phenomenon of decreased tubular reabsorption of sodium after saline administration is related to the salt balance of the subject.

SUMMARY

1. The filtration rate, renal plasma flow and excretion of sodium chloride, potassium and water were followed for eight or more clearance periods (210 minutes) after the intravenous administration of isotonic sodium chloride (1 to 3 liters) or hypertonic sodium chloride (500 cc. of 5 per cent saline) solutions to subjects free of complicating diseases.

2. Neither isotonic nor hypertonic saline solution produced a consistent post-infusion increase in filtration rate or renal plasma flow. Both types of solution, however, increased sodium and chloride excretion irrespective of changes in filtration rate.

3. Whatever significance the filtration rate may have in the excretion of sodium by the human kidney, it appears that other mechanisms are capable of promoting sodium excretion by reducing tubular reabsorption at a constant filtered load.

4. In man, both isotonic and hypertonic saline solutions are retained in the body for long periods, only some 25 per cent, at best, being excreted within three and one-half hours. The recovery of administered electrolyte and water is not appreciably modified by previously loading the body with salt.

5. The human kidney differs from that of the dog in that isotonic and hypertonic saline solutions fail to increase the filtration rate (and renal plasma flow) consistently, a fact suggestively related to the failure of the human kidney to excrete a relatively large fraction of either solution within a few hours.

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THE VELOCITY OF DISTRIBUTION OF SUCROSE BETWEEN PLASMA AND INTERSTITIAL FLUID, WITH REFERENCE TO THE USE OF SUCROSE FOR THE MEASUREMENT OF EXTRACELLULAR FLUID IN MAN [1]

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The following study had two objectives: first, the examination of the question whether or not sucrose can be substituted for inulin in the measurement of the extracellular fluid in man; and second, the determination of how rapidly equilibrium distribution can be attained.

The use of an inulin infusion technique to measure the extracellular fluid in dog and man was initiated by Gaudino, Schwartz and Levitt (1, 2). These investigators used an approximately constant rate of infusion, preceded by a priming infusion, to maintain the plasma concentration of inulin at a constant level for two to 12 hours, during which period it was assumed that uniform concentration throughout the extracellular fluid would be established. After discontinuing the infusion, the total inulin excreted over a period of five hours in the dog or 12 hours in man was determined and, after correction for inulinoid blank in control blood and timed urine samples, the inulin volume of distribution was calculated by dividing total excretion by the plasma concentration at the end of the infusion.[2, 3] The authors concluded that in the dog, uniform distribution was obtained in two hours but they presented no evidence that uniform distribution was not obtained in a shorter time.

Schwartz, Schachter and Freinkel (6), using the same technique, concluded that a maximal volume of distribution had been reached in five normal subjects who had been infused for 3.3, 3.8, 4.5, 7.0, and 7.0 hours, respectively, while in a single individual studied a maximal volume of distribution was not reached in 1.7 hours. The authors concluded that a period of five hours is sufficient to obtain essentially homogeneous distribution, but their data indicate that the critical interval in some individuals may be shorter.

The interval required to obtain homogeneous distribution will depend on the status of the circulation and the dynamics of exchange between the plasma and the interstitial fluid, and it may be anticipated that the equilibrium time will be prolonged under circumstances where the interstitial fluid is increased, or, alternatively, where the plasma-interstitial fluid exchange is sluggish. In so far as diffusion is important in effecting uniform distribution, the equilibrium time may be significantly longer with inulin (mol. wgt. = 5,200) than with a smaller molecule such as sucrose (mol. wgt. = 342).

Sucrose is not quantitatively recovered in the urine after parenteral administration to the dog (7), indicating that it is metabolized in this species. Indeed, Abderhalden and Buadze (cited by Power and Keith [8]) demonstrated the presence of sucrose in dog plasma. However, the evidence indicates that sucrose is not metabolized in man. Keith, Wakefield and Power (9, 10) reported urinary recoveries ranging from 89 to 98 per cent in 12 to 24 hours after administration to subjects without renal disease. Sucrose does not enter the red cells in more than possible traces (9,

[1] We are indebted to the Fourth (New York University Postgraduate Medical School) Medical Division for the privilege of studying patients on that service.

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[2] Gaudino (3) has shown that comparison of the rate of disappearance of inulin from the plasma after a single injection and after prolonged, constant infusion yields data which, on mathematical analysis, conform with the assumption that in the latter circumstance the inulin is uniformly distributed through some volume of body fluid.

[3] Lavietes, Bourdillon and Klinghoffer (4) endeavored to measure the extracellular fluid by calculation based on plasma concentration after the intravenous injection of a single dose of sucrose, but Schachter, Freinkel and Schwartz (5) have shown by studies with inulin that such calculations are unreliable because true plasma-interstitial fluid equilibrium never occurs and virtual equilibrium is only momentary.

10), and it enters the cerebrospinal fluid only in traces (11).

We have examined the 24 hour urinary recovery of sucrose in eight subjects without renal disease, correcting the recovery figure for blank excretion as determined in control, timed urine samples (U_oV, Table I). Sucrose was determined by a modification of the acid hydrolysis method of Lavietes, Bourdillon and Klinghoffer (4), using Nelson's (12) glucose method to determine the liberated glucose, but later Schreiner's (13) modification of the Roe resorcinol method proved more satisfactory. In this method the sucrose is hydrolyzed by the reagent and the liberated fructose reacts with the resorcinol. Plasma was precipitated by Nelson's (12) modification of the Somogyi zinc sulfate method.

Urine was analyzed directly unless protein was present, in which case the urine was precipitated in the same manner as the plasma. The analytical methods were checked by recoveries of aqueous and plasma solutions of known concentrations of sucrose, and in eight determinations in plasma we obtained an average recovery of 99 per cent (range 97 to 101 per cent). Omission of precipitation of protein-free urine did not affect the accuracy of recovery. Blank excretion of a sucrose-like substance under fasting and post-prandial conditions at varying rates of urine flow averaged 0.17 mg./min. (range 0.06 to 0.46) in individuals without renal disease. This does not include three subjects in whom the blank excretion was zero. The results of our experiments are given in Table I and show recovery ranging from 96 to 102 per cent, with an average figure of 99 per cent. We conclude, with Keith and his co-workers, that sucrose is not metabolized in man.

TABLE I

Urinary recovery of sucrose after intravenous injection in man

Subject	Injected	Total recovery	24 hr. U_oV† (calculated)	Corrected recovery	Per cent recovery
	gm.	*gm.*	*gm.*	*gm.*	*per cent*
H. L.	12.42	12.51	0.47	12.0	97
C. F.	6.18	7.07	0.72	6.36	102
N. D.	3.87	4.67	0.75	3.92	101
M. U.	3.92	4.12	0.14	3.97	101
J. G.	5.00	5.07	0.25	4.82	96
D. S.	3.93	4.91	0.95	3.96	100
D. S.*	3.31	4.27	0.99	3.28	99
D. S.*	5.43	6.35	0.97	5.36	99
					Average 99

* These recoveries were carried out using the calibrated infusion technique. The amount injected is obtained from IV-UV.

† 24 hr. U_oV is the calculated 24 hour blank excretion of a sucrose-like substance.

Kruhøffer (14) has shown that the rates of disappearance of sucrose and inulin (determined simultaneously) from the plasma of anesthetized (amytal), nephrectomized rabbits conform with the interpretation that diffusion dominates the distribution of these two solutes in the interstitial space. However, Kruhøffer's data do not show the ultimate volume of distribution of either solute, although they indicate that these volumes are of the same order of magnitude, slightly less than 20 per cent of the body weight. In our opinion, the rates of disappearance of substances from the blood in anesthetized animals must be used with caution since it is possible that they are gravely distorted by reduction of the peripheral circulation.

Methods for the determination of sucrose in the presence of inulin have not proved satisfactory in our hands, so we have determined the volumes of distribution of these two substances in the same individual on separate occasions. Both compounds were administered, after a priming injection, by constant intravenous infusion in order to maintain the plasma concentration approximately constant for five hours, as in the original method of Gaudino, Schwartz and Levitt (1). The total solute in the body at the end of the infusion was determined by a 24 hour urine collection with appropriate correction for blank excretion, and the volume of distribution calculated for the terminal plasma concentration corrected for plasma protein. The results of comparative studies on four edema-free subjects are given in Table II. The ratio sucrose/inulin space averaged 0.972 (range 0.936 to 1.032). In view of the facts that the determinations are not simultaneous and that the above ratio is less rather than greater than 1.0 we believe that our data warrant the use of sucrose in man for the determination of the extracellular fluid volume.

Determination of time required for attainment of maximal volume of distribution. The novel feature in this technique is the calculation of the virtual volume of distribution at any instant after the start of a constant intravenous infusion without the use of a priming injection.

The virtual volume of distribution of any non-metabolized solute, x, at any time, t, is given by the expression

$$V_x = \frac{\Sigma IV - \Sigma UV}{P_t}, \qquad (1)$$

where V_x is the virtual volume of distribution (in cc.), ΣIV is the total quantity of solute that has been infused (in mg.), ΣUV is the total quantity of solute that has been excreted in the urine (in mg.)[4] and P is the concentration of the substance in the plasma (here neglecting the plasma water content for simplicity of discussion).

Just after the infusion has been started the solute will be largely restricted to the plasma and P will be large, relative to the concentration in the extracellular fluid, and V_x will consequently have a small value. If the rate of excretion increases in direct proportion to P (as in the case of sucrose and inulin), with the passage of time, UV (in mg./min.) will increase from zero and approach IV (in mg./min.), a condition that will be fulfilled only when P has become constant, which will not occur until a homogeneous distribution of solute has been reached. During this period of equilibration, V_x will increase and approach a maximal value which will be identical with the volume of distribution to be expected at equilibrium, *i.e.*, when P has been maintained constant for some period of time.

If the rate of mixing between plasma and interstitial fluid were instantaneous and if the relations between the circulation and the kidney were such as to permit instantaneous equilibration between UV and P, then after the infusion is started IV and UV would also come into equilibrium instantaneously, P would instantaneously become constant, and V_x would instantaneously reach its maximal value. Such, of course, is not the case because of delay in escape from the plasma, delay in movement into the remote recesses of the interstitial fluid, and particularly because of delay in attaining equilibrium across the kidneys.[5]

[4] We reserve the expressions IV and UV to indicate rates of infusion or excretion (mg./min.), in accordance with established convention in renal physiology, and therefore designate the total quantities infused or excreted at any time as ΣIV and ΣUV.

[5] It may be noted that Michie and Michie (15) have shown that an average of 25 minutes is required to bring the pelvic urine into excretory equilibrium with the arterial plasma.

It is of critical importance in this method that the composition of the infusion be known with great accuracy and that the rate of infusion be kept very constant and known exactly, because slight errors in IV become compounded with time in the calculation of ΣIV and therefore in all derivative calculations. (As calculated from a typical experiment, a variation of 10 per cent in the rate of infusion will produce an error of 5 per cent in V_s after 15 minutes at a time when $\Sigma UV = 0.14\ \Sigma IV$.) Sucrose was infused in a 9.5 to 10.5 per cent solution with a Bowman constant infusion pump,[6] delivering the infusion fluid at a rate of 0.665 to 0.700 cc./min. When this pump is powered through a Sola constant voltage transformer, the rate of infusion, as checked at 30 and 60 minute intervals, remains constant with an accuracy of ± 2 per cent. The infusion reservoir consisted of a series of 20 and 40 cc. glass bulbs with calibrated stems connected by ground glass joints, permitting the rate of infusion to be checked from time to time. The volumes delivered by the bulbs were found to be reproducible within ± 1 per cent despite repeated autoclaving. Care must be taken to keep them chemically clean so that drainage will be complete. It is essential that a continuous check of this type be kept on the rate of the pump throughout the infusion.

A control sample of blood and a timed urine sample were collected before starting the infusion in order to determine the plasma blank and the rate of excretion of urine blank. Urine samples were collected by an indwelling catheter and the bladder was rinsed with saline and emptied with air at the end of each urine collection period. Error occasioned by renal dead space was minimized by

[6] Manufactured by the Process & Instruments Corporation, 60 Greenpoint Avenue, Brooklyn, New York.

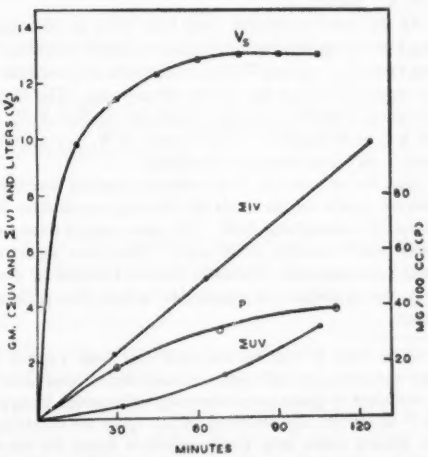

FIG. 1. TYPICAL EXPERIMENT SHOWING THE VIRTUAL VOLUME OF DISTRIBUTION OF SUCROSE, V_s, DURING INFUSION AT A CONSTANT RATE

TABLE II

Comparative volumes of distribution of sucrose and inulin in four edema-free subjects, determined on separate occasions by the five hour infusion method

Patient.....	F. R.		R. F.		S. D.		R. M.	
	liters	*per cent body weight*	*liters*	*per cent body weight*	*liters*	*per cent body weight*	*liters*	*per cent body weight*
Sucrose space	10.20	16.9	11.81	23.8	10.14	15.2	10.80	15.6
Inulin space	10.40	17.3	12.56	25.3	10.82	16.2	10.47	15.2
Sucrose/inulin	0.981		0.939		0.936		1.032	

maintaining water diuresis, generally at the level of 3 cc./min. in adults and at comparable rates in children. The timing of blood samples, urine collection and infusion rates was noted to the nearest quarter of a minute. The amounts of sucrose used were not such as to produce any significant change in the osmotic pressure of the plasma.

The results are analyzed in the following manner. The total quantity of sucrose infused (ΣIV) at successive intervals is plotted against elapsed time. These points should fall on a straight line (see ΣIV, Figure 1). The total amount of sucrose excreted (ΣUV) is calculated for each successive urine sample (see ΣUV, Figure 1). These points should fall along a smooth curve and serve as a check upon the accuracy of urine collection and analysis. The plasma concentration of sucrose, as determined at various intervals, is also plotted against time (see P, Figure 1).[7] With frequent sampling of plasma it is possible to check for deviations produced by analytical error. The virtual volume of distribution of sucrose ($\mathbf{V}_s$) (uncorrected for plasma water) at any time during the infusion is obtained by inserting the appropriate interpolated values for ΣIV, ΣUV, and P in equation 1 (see $\mathbf{V}_s$, Figure 1).

At the rates of infusion used here (60.0 to 80.0 mg./min.) the error involved in neglecting blank excretion is less than 4 per cent of ΣUV at 60 minutes and less than 1 per cent of ΣUV at the end of 120 minutes. The blank excretion is usually less than 1 per cent of ΣIV–ΣUV at the end of 60 minutes. Thus errors in $\mathbf{V}_s$ are minimal even if the blank excretion is omitted.

Selection of patients. The patients examined here were selected solely on the basis of showing no obvious increase in extracellular fluid. The ages ranged from two years and 10 months to 60 years. Data on a nephrotic child two years and 10 months old are included to show that the procedure is practicable within the pediatric range.

[7] The term P may be corrected for water content in this operation, but this involves superfluous labor and it is sufficient to make the preliminary calculations in terms of P as analytically observed and to apply the correction for plasma water only to the definitive figure for maximal volume of distribution. This correction is effected by multiplying the final volume of distribution by the fraction of water in the plasma.

RESULTS

A typical experiment illustrating the application of the above method (which for brevity we will designate the calibrated ΣIV–ΣUV method) is shown in Figure 1. It will be observed that the virtual volume of distribution of sucrose ($\mathbf{V}_s$) attained a practically constant value in this patient in 70 minutes. In 14 patients without edema (Table III) the time required to attain a constant value ranged from 30 to 120 minutes and averaged 75 minutes.

The next problem is to what extent this volume of distribution approaches the maximal volume of distribution. This point was examined by comparing the results obtained by the calibrated ΣIV–ΣUV method with those obtained by using a prolonged equilibration time, as in the method of Gaudino, Schwartz and Levitt (which we will designate the total urine recovery method). At the end of three hours of infusion, using the calibrated ΣIV–ΣUV method, a priming infusion of sucrose was administered and the infusion continued for an additional five hours with the maintenance of a constant plasma concentration as demonstrated by plasma samples taken at the end of two, three, four, and five hours. At the end of the five hour period the infusion was discontinued and urine was quantitatively collected for the next 24 hours. The volume of distribution of sucrose was calculated from the amount of sucrose recovered in the urine and the plasma concentration at the termination of the infusion.

As shown in Table IV, the results obtained by the two methods are in good agreement in three subjects. It follows that, so far as these subjects are concerned, the rate of mixing between plasma and extracellular fluid is very rapid and that, despite continuing increase in P, this term is, within the limit of technical error, identical with the con-

TABLE III

Maximal volume of distribution in 14 subjects measured by the calibrated infusion method

Patient	Sex	Age	Weight	Diagnosis	Approx. time required for maximal volume of distribution	$\frac{UV}{IV} \times 100$	Maximal volume of distribution		Sodium space	
		yrs.	*kg.*				*liters*	*per cent body weight*	*liters*	*per cent body weight*
H. C.	M	60	74.1	Normal	120	31	13.8	18.8	19.1	25.8
T. G.	M	36	88.4	Normal	30	12	14.3	16.2	18.6	21.0
J. M.	M	34	85.9	Normal	60	23	16.4	19.1	—	—
S. R.	M	14	47.8	Normal	90	34	7.8	16.3	—	—
D. R.	M	55	64.4	Hyperthyroidism	80	22	11.5	17.9	—	—
J. P.	F	50	45.9	Hyperthyroidism	120	30	8.4	18.6	—	—
R. N.	F	46	45.0	Thyroid malignancy	105	13	10.2	22.6	13.9	30.9
L. P.	F	65	61.3	Thyroid malignancy	60	28	8.7	14.4	12.9	21.0
M. P.	F	56	55.9	Thyroid malignancy	105	26	11.4	20.4	14.9	26.7
E. L.	M	48	70.0	Polycythemia vera	30	10	12.3	17.5	17.2	24.6
S. H.	M	$2\frac{10}{12}$	16.9	Nephrosis	30	3	3.7	22.0	4.7	28.0
R. F.	M	14	49.6	Acute rheumatic fever	70	29	12.2	24.6	—	—
M. U.	F	27	43.4	Rheumatoid arthritis	105	43	9.4	21.6	—	—
S. D.	M	18	66.7	Acute rheumatic fever	45	20	10.5	15.7	—	—

centration in all portions of the extracellular fluid. Since constancy of V_s was attained in all subjects listed in Table III, we presume that this conclusion is also applicable to them.

Where a major part of the infused sucrose is excreted, the term ΣUV will be large relative to ΣIV and the accuracy of any calculation based on the difference, ΣIV–ΣUV, will be diminished. Therefore, we have shown in Table III the fraction of the administered sucrose excreted at the time when the virtual volume of distribution first became constant. This figure ranges from 3 to 43 per cent. Thus the term (ΣIV–ΣUV) is always greater than 50 per cent of ΣIV and the error from this source is kept to a minimum.

Simultaneous measurements of the volume of distribution of sucrose and Na^{24} were made in seven patients (Table III). It is known that sodium has a variable intracellular distribution and that the sodium space is always larger than the volume of extracellular fluid. This was the case in all patients studied here.

The volume of distribution of sucrose as determined by the ΣIV–ΣUV method and corrected for plasma water ranged from 14.1 to 24.6 per cent and averaged 19 per cent of the body weight. These studies were carried out in patients with a variety of pathological disturbances, however. The volume of distribution of sucrose in the four subjects whom we have listed as "normal" ranges from 16.2 to 19.1 per cent of the body weight (average 17.6 per cent), figures that agree with those reported by previous investigators using the total urine recovery method.

TABLE IV

Comparison of sucrose space in three individuals as measured by the calibrated infusion method and the total urine collection method

Patient.....	R. F.		M. U.		S. D.	
	liters	*per cent body weight*	*liters*	*per cent body weight*	*liters*	*per cent body weight*
Sucrose space Calibrated infusion method with changing plasma concentration	12.22	24.6	9.40	21.6	10.53	15.7
Sucrose space Five hour infusion method with constant plasma concentration and 24 hour urine collection	11.81	23.8	8.82	20.4	10.14	15.2

DISCUSSION

The method described here affords a more practical approach to the measurement of extracellular fluid than that presented by the five hour equilibration and the total urine collection method. It has the advantage that it can be extended to patients in the pediatric range and to almost all adults, and in general a valid determination can be completed within 180 minutes. If approximately uniform distribution throughout the body fluids is not attained, the method reveals this fact by the circumstance that the virtual volume of distribu-

tion, as here calculated, will not have attained a maximal value. Seriatim determinations during the three hours of infusion therefore afford a safeguard against sluggish mixing. The method is not applicable in its present form to subjects in the edematous state without prolongation of the infusion.

SUMMARY

We have confirmed the observations of others that sucrose is not metabolized in man (as it is in the dog) and in four patients we have demonstrated by the five hour infusion-equilibration technique plus 24 hour urine collection that sucrose has the same volume of distribution, within the limits of technical error, as does inulin. Because the difference in molecular weight between sucrose and inulin may make a significant difference in the rate of equilibration within the interstitial fluid, we have substituted sucrose for inulin in a study of the kinetics of mixing between plasma and interstitial fluid by means of a calibrated infusion technique in which a priming injection is omitted.

The results obtained on four normal subjects and 10 patients with a variety of pathological disturbances show that the mixing process is of such a nature that within 30 to 180 minutes after the beginning of a sucrose infusion (without a priming dose), the plasma has for practical purposes come into equilibrium with the interstitial fluid. The volume of distribution of sucrose measured when apparent equilibrium distribution has been reached agrees in three patients with the volume of distribution as measured by the five hour infusion-total recovery method. This approach to equilibrium is attained despite the fact that the plasma concentration is still increasing, a circumstance that indicates relatively rapid mixing between plasma and interstitial fluid.

The method has the advantage that it reveals failure to attain equilibration between the plasma and extracellular fluid in any particular patient.

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INTRACELLULAR WATER IN MAN [1]

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Measurements of intracellular water, calculated as the difference between the volumes of distribution of antipyrine and inulin, have been reported by Berger and associates (1) in four patients with chronic disease. Comparable data are not available on normal subjects and the present study was undertaken to make such data available.

METHODS

Total body water was determined as the volume of distribution of antipyrine,[2] corrected for the amount metabolized, by the method of Soberman and co-workers (2). Soberman and his associates report good agreement in this measurement as made with antipyrine and with deuterium oxide in six normal males. However, these investigators assumed that maximal distribution of antipyrine was reached in two hours and they consequently used the plasma concentrations determined at two, three and five hours for extrapolation to zero time in correcting for metabolism. In view of the fact that sucrose, which is confined to the extracellular fluid, may not reach maximal distribution in two hours in some non-edematous subjects (3), we have preferred to base our extrapolation on the plasma concentration at three, four and five hours, and, where possible, to include the concentration at seven or eight hours.

Extracellular fluid was measured by the maximal volume of distribution of sucrose as determined by the calibrated infusion technique (3). Intracellular water was determined as the difference between the antipyrine and sucrose volumes of distribution. Cellular mass was calculated as the difference between body weight and sucrose volume of distribution.

The subjects were patients ranging in age from 13 to 60 years who had been hospitalized for elective surgery or routine physical examination.

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We are indebted to the Fourth (New York University Postgraduate Medical School) Medical and Surgical Divisions of Bellevue Hospital for the privilege of studying patients on these services.

[2] The antipyrine used in these studies was supplied in ampoule form by Eli Lilly and Company, Indianapolis 6, Indiana.

RESULTS AND DISCUSSION

Table I presents the simultaneous measurements of total body water and extracellular fluid in 11 male subjects. Calculated values for the volume of intracellular water, intracellular water as per cent of body weight, intracellular water as per cent of body water, and intracellular water as per cent of cellular mass, are indicated for each subject. Intracellular water averaged 40.6 per cent of body weight (range 34.7 to 48.0) and 70.2 per cent of total body water (range 64.9 to 74.5). Water comprised on the average 50.3 per cent (range 41.8 to 59.6) of cellular mass.

In the six males studied by Soberman and his co-workers (2) the average antipyrine and deuterium oxide space was 54.6 and 55.8 per cent, respectively, of the body weight. Steele and colleagues (4) report the average antipyrine space as 52.7 per cent of body weight in 51 normal males, while Schloerb and associates (5) report the average deuterium oxide space as 61.8 per cent of the body weight in 18 normal males. Our antipyrine figure based on 11 males is 58.1 per cent (range 51.8 to 64.5), a figure that is in better agreement with the deuterium oxide data cited above. We believe that our figure is higher than that reported by Steele and co-workers (4) because we have neglected the two hour plasma concentration in favor of concentrations from three to eight hours.

Schwartz, Schachter and Freinkel (6) have reported an average inulin space of 16.3 per cent of body weight in six males. The sucrose space in the present series of 11 males averages 17.5 per cent of body weight. This agreement supplements the data previously presented (3) that sucrose and inulin are restricted to a similar fluid compartment.

Intracellular water in the four patients studied by Steele and his co-workers (4) averaged 40.7

TABLE I

Intracellular water in 11 male subjects

Patient	Age	Height	Weight	Total body water		Extracellular fluid		Intracellular water			
	yrs.	*in.*	*kg.*	*liters*	*per cent body weight*	*liters*	*per cent body weight*	*liters*	*per cent body weight*	*per cent body water*	*per cent cell mass*
C. E.	22	71	73.6	44.8	60.8	14.1	19.1	30.7	41.7	68.5	51.6
A. M.	41	72	84.5	43.7	51.8	14.4	17.1	29.3	34.7	67.0	41.8
R. M.	58	72	85.0	47.4	55.8	16.7	19.6	30.7	36.1	64.9	45.0
J. T.	13	54	37.2	20.3	54.6	5.2	14.0	15.1	40.6	74.5	47.2
K. O.	43	67	69.6	38.4	55.3	11.3	16.2	27.1	39.0	70.6	46.5
T. E.	36	68	77.0	49.6	64.5	12.6	16.3	37.0	48.0	74.5	57.5
T. G.	36	73	88.4	53.4	60.2	14.3	16.2	39.1	44.3	73.5	52.8
R. M.	29	69	70.5	42.7	60.5	10.9	15.5	31.8	45.1	74.4	59.6
H. C.	60	68	74.1	45.5	61.4	13.9	18.8	31.6	42.6	69.5	52.5
L. C.	14	60	44.3	25.0	56.4	8.7	19.7	16.3	36.8	65.1	45.8
W. J.	30	63	64.1	37.4	58.2	12.5	19.5	25.9	38.7	69.1	50.2
Average					58.1		17.5		40.6	70.2	50.3
Range					51.8 to 64.5		14.0 to 19.7		34.7 to 48.0	64.9 to 74.5	41.8 to 59.6

per cent of body weight (range 29.3 to 47.0), as compared with an average of 40.6 per cent (range 34.7 to 48.0) in this study.

SUMMARY

Total body water and extracellular fluid have been measured simultaneously by the antipyrine method and the calibrated sucrose infusion technique in 11 normal male subjects. Intracellular water has been calculated as the difference between total body water and extracellular fluid.

Intracellular water averages 40.6 per cent of body weight (range 34.7 to 48.0), 70.2 per cent of total body water (range 64.9 to 74.5) and 50.3 per cent of cellular mass (range 41.8 to 59.6).

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TOTAL RED CELL VOLUME, PLASMA VOLUME, AND SODIUM SPACE IN CONGESTIVE HEART FAILURE[1]

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A large volume of literature has accumulated in the past 15 years dealing with the importance of hypervolemia in the pathologic physiology of congestive heart failure. Most of the series of blood volumes reported to date on this question have been measured with the dye T-1824 (1–4), although other dyes have been used (5–7). The errors in the dye methods have been repeatedly pointed out (8–16). Chief among these are the early and significant disappearance of the dye-albumin complex into: (*1*) the lymphatic system; (*2*) the reticulo-endothelial system; and (*3*) the bile. In reality, therefore, the dye space is not synonymous with plasma volume but includes a part of the volume of the lymphatic system, biliary and reticulo-endothelial systems. The degree to which these latter will be included depends on: (*1*) individual mixing and diffusion factors, and (*2*) the time at which one concludes from the dilution curve that mixing has become complete.

In most of the above-mentioned studies, patients with definite signs of congestive failure have been found, almost without exception, to have significant elevation of their blood volume, amounting in some cases to as much as 300% above established normal values for the method. These likewise have been noted to return to the established normal levels upon return to the compensated state. However, it should be noted that Wollheim (*7*), using a modification of the dye method of Keith and Rowntree, (17), noted two types of congestive failure; the first with high and the second with low blood volumes. In both groups the blood volume returned toward normal as compensation was achieved.

The relative magnitude and time relationships of total red cell volume and the plasma volume during congestive failure were also examined (1–4). Gibson and Evans (1) found that total red cell volume was elevated to a greater degree than plasma volume. In contrast, Seymour and his associates (*2*) found that the plasma volume rose more than did the total red cell volume. Other groups (3, 4) noted an equal rise in these components of blood volume. There was general agreement, however, that plasma volume was definitely more labile than total red cell volume, falling considerably more rapidly as compensation proceeded. This resulted in a temporary rise of the hematocrit, which returned to normal as the excess red blood cells were destroyed.

The relation between the severity of failure, as indicated objectively by venous pressure, and degree of hypervolemia represents a point of disagreement among the above-mentioned investigators. Gibson and Evans (1) found a direct and definite correlation between increasing venous pressure and rising blood volume, which Meneely and Kaltreider (3) did not confirm.

Since the development of methods utilizing red cells labeled with P^{32} (18) or $Iron^{59}$ (19), blood volume determination would appear to be on a theoretically sounder basis, the chief advantage being the localization of the labeled red blood cells in the vascular system and a consequent plateau level of radioactivity for a finite period of time (approximately one hour with P^{32}) (20) following mixing. During this time a sample can be withdrawn to determine accurately the degree of dilution which has taken place.

There is one possible objection to the determination of the total blood volume by the tagged red cell method; namely, that the hematocrit in peripheral veins may differ significantly from the "total body" hematocrit. Good evidence is lacking on this point, and the degree of difference depends on the method of approach to the problem. Ebert

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and Stead (21) removed an ordinary venous sample from the antecubital vein for hematocrit, hemoglobin and protein content, then applied a tourniquet to the upper arm at 300 mg. Hg pressure, removed all the available blood, this blood presumably representing that in the larger vessels. Thereafter, an Esmarch bandage was applied to the forearm from the wrist up and any remaining blood in the arm was thus "milked" out. This blood was thought to come from the minute vessels of the arm. When compared, the hemoglobin concentration of the last samples were from 0.8 gm. to 1.89 gm./100 cc. less than the original venous sample. They concluded that venous blood is richer in cells and poorer in plasma than the blood contained in minute vessels. From their figures, the value of small vessel hematocrit is approximately 10% lower than that in the peripheral veins. Furthermore, the total estimated amount of blood in the minute vessels is from 10% to 17% (22, 23). Therefore, the maximum difference between venous hematocrit and total body hematocrit brought about by the blood in the minute vessels could not exceed 1.5% unless a very large percentage of the blood was in the capillaries. The concept that the "total body hematocrit" differs from the venous hematocrit (24–27) has largely arisen because the total red cell volume as determined by labeled red cells has been shown consistently to be less than the total red cell volume as calculated from the plasma volume determined with T-1824 and the venous hematocrit. However, as pointed out above, the dye methods are subject to error, giving falsely high plasma volumes and in turn falsely high calculated red cell volumes. In fact, even the various modifications of the dye method by the Louisville group (28–30) were not satisfactory in that the total red cell volume as determined by the dye was still higher than when methemoglobin labeled red cells were used. Therefore, the total body hematocrit concept, at least from this standpoint, rests on questionable experimental evidence. Since the total body hematocrit depends upon the plasma volume, a falsely high plasma volume calculation would lead to a falsely low total body hematocrit.

$$\left(\text{Total body hematocrit} = \frac{\text{Total red cell volume}}{\text{Total plasma} + \text{total red cell volume}}.\right)$$

From the available evidence we do not believe that the total body hematocrit differs significantly from the venous hematocrit. We therefore believe the labeled red cell method for determining blood volume to be accurate. No positive data have been produced to support this concept. But the weight of evidence against varying hematocrits, when viewed as a whole (16), certainly lends credence to it.

Nylin and Hedlund (20) studied seven patients in congestive failure using P^{32} labeled red cells; their results are not in complete agreement with the previous studies with the dyes. The data of these authors differed from the dye studies in that with primary left-sided failure with pulmonary congestion and high venous pressure, blood volume was found to be practically normal and to remain practically constant with return of compensation. They found no correlation between venous pressure and blood volume in their cardiac patients. Recently Ross, Baka and Freis (31) have studied a group of patients with congestive failure with P^{32} labeled cells, and found that there was no significant increase in circulating blood volume in patients with congestive failure as compared to controls. The types of cardiac abnormalities studied were not stated in this abstract.

METHODS

The blood volume was determined with P^{32} labeled cells in 27 patients with congestive heart failure by the method of Hevesy and Zerahn (18) as modified in this laboratory (32, 33).[3] With labeled red blood cells, the total red cell volume is determined and is independent of the hematocrit. The plasma volume and blood volume are calculated from the total red cell volume and the venous hematocrit. Since, as pointed out above, it is unlikely that venous hematocrit differs significantly from the "total body hematocrit," the calculation of blood volume and plasma volume by this method should not lead to error.

Serum sodium was determined by the zinc uranyl acetate method (34). The normal range of serum sodium concentration using this method is 135–148 meq./liter. For the determination of total exchangeable body sodium, patients were given approximately 100 μc. of Na^{24} and the radioactivity of a plasma sample 18 hours after adminis-

[3] Blood samples for determination of dilution were taken 20 minutes after administration of P^{32} labeled cells. Nylin and Hedlund (20) have shown that mixing of red cells is complete well within this time in patients with congestive failure.

tration was determined in the same manner as were the P^{32} samples (32).

$$\text{Total exchangeable body sodium space} = \frac{\text{Disintegration/min. } Na^{24} \text{ injected} - \text{Disint./min. } Na^{24} \text{ excreted in urine}}{\text{Disint./min. } Na^{24}\text{/cc. of plasma}}$$

This calculation assumes a homogeneous concentration of the sodium. Since sodium is not homogeneously distributed but is present in high concentration in bone and in low concentration in cells, the value obtained by calculation is virtual rather than actual. However, "sodium space" measurements are of considerable value when used comparatively. Total exchangeable sodium is then determined by the following equation, assuming that at the end of 18 hours the specific activity of Na is uniform throughout the body.

$$\text{Serum sodium concentration in meq./liter} \times \text{"Sodium space" in liters} = \text{Total sodium in meq.}$$

Where the 18-hour plasma samples contained both Na^{24} and P^{32}, the following method was used to delineate the amount of activity of each:

(*1*) The 18-hour plasma sample was counted initially, and one equation set up: $Na^{24} + P^{32}$ = initial count.

(*2*) The same sample was counted 24 hours later, and a second equation set up: % remaining Na^{24} + % remaining P^{32} = count at 24 hours.

The per cent remaining of each isotope at any given time was calculated from their well-established physical half-lives. There are then two simultaneous linear equations with two unknowns which can easily be solved for both unknowns.

The criteria for heart failure are the usual; namely, evidence of cardiac abnormality by history, physical examination and/or laboratory means. In addition, the presence of all or several of the following were required: dyspnea, orthopnea, cyanosis, pulmonary congestion and edema, peripheral edema, and fluid in the thoracic or abdominal cavities. Details of therapy are not outlined for each patient because Table III describes the exact clinical status of the patient on the day the blood volume was determined, as evaluated by examination at that time.

The symptoms and physical signs of each patient are given in Table III. The data are expressed in terms of "wet" and "dry" cc./kilo. Wet weight includes edema fluid. Dry weight indicates body weight when patient had returned to partial or fully compensated state. Where only one determination was done, we have arbitrarily subtracted 6 kilos and used the resulting figure as "dry" weight. The expression of volumes as cc./kilo of "dry" weight was used in order to avoid getting falsely low values, expressed as cc./kilo, because of excess weights. Admittedly, a certain percentage of patients will have more than 6 liters of edema fluid, so that our correction factor will be inadequate. However, even assuming they contain 12 liters excess, the values expressed in cc./kilo in a 70-kilo individual would be only 8.5% too low. Furthermore, the majority of patients studied had minimal to moderate edema, only four being classed as 4+ in degree. Warner and his co-workers (35) have recently observed the correlation between edema and *excess* "sodium space." They found that 1+ edema in an average individual (1.73 m^2) corresponded to an excess of roughly 3 liters while 4+ edema represented approximately 13 liters, with gradations as expected between these limits. These figures further validate our 6-

TABLE I

Group with normal blood volumes

Name	Date	Age	Weight		Total blood volume (*cc.*)	Total blood volume (*cc./kilo*)		Total red cell volume (*cc./kilo*)		Plasma volume (*cc./kilo*)		Hemato-crit
			Wet	Dry		Wet	Dry	Wet	Dry	Wet	Dry	
*R. W.	8-23-50	40	—	61.4	4,236	—	68.9	—	28.1	—	40.0	41
M. S.	7-25-50	74	78.6	72.6	3,598	45.8	49.6	26.1	28.2	19.2	20.8	51
1 C. R.	8-17-50	38	68.6	—	3,740	54.5	—	23.3	—	30.7	—	43
2 C. R.	8-31-50	38	—	54.5	4,199	—	77.0	—	33.1	—	43.2	43
*H. R.	7- 6-50	59	—	81.8	3,755	—	45.9	—	20.2	—	25.2	44
C. P.	7-18-50	71	59.5	53.5	3,481	58.5	75.6	24.6	31.8	33.3	43.1	47
*W. M.	11-17-50	84	—	81.8	5,387	—	65.8	—	26.3	—	38.9	40
*F. G.	7-26-50	74	—	102.3	4,239	—	41.4	—	14.5	—	26.5	35
M. Gr.	8- 9-50	53	61.4	55.4	4,354	70.9	79.2	29.8	33.2	40.4	46.0	47
M. G.	5-22-50	47	64.5	59.5	3,380	52.4	58.8	27.7	30.3	24.7	28.5	53
1 A. G.	6-14-50	62	73.1	—	4,605	62.9	—	32.1	—	30.2	—	51
2 A. G.	6-22-50	62	—	68.1	5,196	—	76.3	—	38.1	—	37.4	50
1 *S. C.	12- 7-49	34	—	52.2	2,694	—	51.5	—	20.6	—	30.0	40
2 S. C.	8-18-50	34	—	52.2	3,012	—	57.9	—	24.8	—	32.3	43
1 T. A.	5-12-50	62	106.3	—	4,084	37.7	—	23.4	—	14.2	—	61
2 T. A.	9-27-50	62	—	99.1	6,793	—	68.5	—	34.9	—	32.9	51
Mean values		58.3	73.1	68.8	3,962.7	54.7	62.8	26.7	28	27.5	34.2	44.5

* Patient too ill to be weighed. Therefore, either the patient's usual weight prior to illness was used; or, when available, the weight was determined after recovery.

TABLE II

Group with high total red cell volume, plasma volume or total blood volume

Name	Date	Age	Weight		Total blood volume (cc.)	Total blood volume (cc./kilo)		Total red cell volume (cc./kilo)		Plasma volume (cc./kilo)		Hematocrit
			Wet	Dry		Wet	Dry	Wet	Dry	Wet	Dry	
A. Patients with Rheumatic Valvulitis												
1 R. S.	9-14-50	50	56.4	—	5,113	90.7	—	41.7	—	49.0	—	46
2 R. S.	10-16-50	50	—	50.9	4,500	—	88.4	—	41.4	—	45.9	47
L. S.	11-21-50	61	48.2	42.2	4,255	88.3	101.0	37.1	42.3	51.2	58.7	42
I. R.	12- 1-50	34	43.6	37.6	4,505	103.3	120.0	46.5	54.0	56.8	66.0	45
P. D.	11-16-50	47	70.0	64.0	6,768	96.7	100.6	46.4	50.7	49.7	49.9	48
C. E.	1-29-51	45	60.0	54.0	4,969	82.8	91.8	40.6	45.1	41.4	46.0	49
C. C.	1- 2-51	48	57.7	51.7	5,000	86.7	96.7	39.8	44.5	45.8	51.2	46
M. B.	1-29-51	46	47.3	41.3	3,447	73.0	83.4	35.7	40.6	36.4	41.7	49
Mean		47.2	56.4	48.8	4,865	88.7	97.4	41.1	45.5	47.1	51.3	46.4
B. Nonrheumatic Patients												
S. W.	8-31-50	43	55.5	49.5	3,377	60.8	68.0	17.0	19.5	42.6	47.6	28
W. N.	12- 5-50	64	60.0	54.0	3,706	61.8	68.5	14.8	16.5	46.3	52.0	24
H. M.	8- 3-50	44	81.8	75.8	6,319	77.2	83.3	19.4	20.8	57.8	62.5	25
D. M.	8- 2-50	61	54.5	49.5	3,533	64.8	73.0	37.0	41.5	27.8	31.5	57
1 M. K.	7-13-50	34	51.7	—	4,469	86.4	—	27.7	—	57.9	—	32
2 M. K.	7-27-50	34	—	45.9	4,588	—	99.9	—	39.0	—	60.9	39
*M. H.	12- 8-50	83	—	39.5	2,629	—	66.5	—	37.2	—	28.6	56
*A. G.	11-30-50	74	—	59.5	4,421	—	74.3	—	18.5	—	54.9	25
B. K.	12- 8-50	70	49.1	43.1	4,417	90.0	102.5	40.5	46.2	48.6	55.4	45
Mean		57.5	60.6	53.4	4,065	73.4	79.5	26.0	29.9	46.8	49.1	32.9

* See footnote to Table I.

kilo correction factor for patients, most of whom had minimal to moderate edema.

Venous pressures were determined by measuring the height of a column of saline in a spinal fluid manometer, the arm being leveled by means of external landmarks at a point approximating the entrance of the superior vena cava into the right auricle. Arm to tongue circulation times were determined with 5 cc. of "decholin."

RESULTS

The results are summarized in the Tables I–III and Figures 1–3.

DISCUSSION

For purposes of discussion it should be noted that the normal total red cell volume and plasma volume values in males and females expressed in cc./kilo are as follows (33, 36): In males the total red cell volume averages 29.9 (range 22.8 to 35.8) and the plasma volume, 38.7 (range 32.6 to 45.1). In females the total red cell volume averages 27.0 (range 21.1 to 32.7) and the plasma volume, 37.0 (range 27.3 to 46.6).

Table I includes all patients who had entirely normal or low total red cell volume, plasma volume and blood volume while in failure. Twelve patients are in this group, or 44.5% of the total; six are males and six females. This group can be divided into three clinical categories: *a*) Primarily

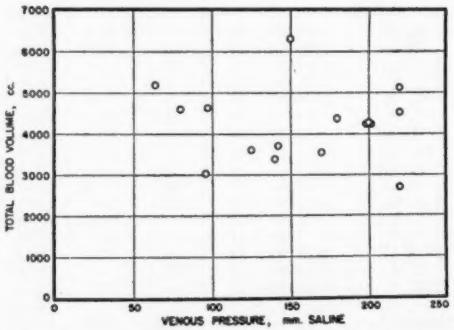

FIG. 1. RELATIONSHIP OF BLOOD VOLUME TO VENOUS PRESSURE, IN TOTAL CC.

TABLE III

	R. W.	M. S.	C. R. (1)*	C. R. (2)*	H. R.	C. P.	W. M.	F. G.
Age	40	74	38	38	59	71	84	74
Sex	M	F	F	F	F	M	M	M
Diagnosis	HCVD†	HCVD Diabetes mellitus	HCVD	HCVD	Myocardial infarction ASHD‡	ASHD	Myocardial infarction HCVD	ASHD Myocardial infarction
B. P.	138/120	190/100	144/102	—	110/60	150/80	214/118	140/105
Pulse	90	92	100	66	60 Sl. cyanosis	80	88 Cyanosis	90
Respiration	30	28	26	18	28 Shallow	30	34	32
Heart	Enlarged, Grade III mitral systolic, gallop rhythm	Enlarged, regular. Grade III mitral systolic murmur	Enlarged, gallop rhythm. No murmurs	Enlarged, gallop rhythm. No murmurs	Sl. enlarged, frequent extra systoles, tones distant	Enlarged, regular. Soft mitral murmur	Enlarged, regular, distant tones	Enlarged, regular, distant tones
Lungs	Basal rales right hydrothorax	Rales throughout both lung fields	Clear	Clear	Crepitant rales both bases	Rales lower 2/3, both lungs	Moist rales lower 2/3, senile emphysema	Crepitant rales, both bases
Liver	Two fingers below costal margin	0	Questionable due to ascites	Two fingers below costal margin	0	Questionable due to dyspnea and orthopnea	Not felt	Four fingers below costal margin
Ascites	0	0	Very marked	None	0	0	0	0
Peripheral edema	0	++ Feet and ankles	None	None	0	Extensive sacral genitalia	Minimal sacral	0
Duration of illness	Three weeks. Repeated failures one year	Two weeks	Nine days. Repeated failures past three years	Two weeks later	Three weeks progressive. EKG myocardial infarction	Gradual. One year. Rapid progress— 3–4 weeks	Acute myocardial infarction— 2 days	Increasing dyspnea one week. Infarction of myocardium 1 day
V. P.	200	125	Not done	Not done	Not done	Not done	Not done	200

TABLE III—*Continued*

	M. Gr.	M. G.	A. G. (*1*)	A. G. (*2*)	S. C. (*1*)	S. C. (*2*)	T. A.	R. S. (*1*)
Age	53	47	62	62	34	34	63	50
Sex	F	F	M	M	F	F	M	M
Diagnosis	HCVD	RhHD.§ Auric fibrillation	HCVD	HCVD	HCVD due to chronic glomerulonephritis	HCVD due to chronic glomerulonephritis	HCVD Obesity	RhHD with mitral stenosis and constrictive pericarditis
B. P.	180/120	110/70	200/170	—	260/160	210/130	160/100	110/80
Pulse	96	128	120 Sl. cyanosis	80	130	80	80	90
Respiration	24	26	24	20	24 Labored	20	26	22
Heart	Very large. Frequent extra systoles, Grade III aortic murmur	Enlarged, fibrillating, loud mitral systolic murmur	Enlarged, regular, with mitral systolic and diastolic murmurs	Enlarged, regular, with mitral systolic and diastolic murmurs	Enlarged, regular	Enlarged, regular	Enlarged, regular. No murmurs	Enlarged, fibrillating, mitral systolic and diastolic murmurs
Lungs	Systolic murmur. Crepitant rales throughout both lungs	Crepitant and moist rales lower halves	Bilateral crepitant basal rales	Clear	Loud bubbling rales throughout	Clear	Moist rales left base	Crepitant and moist rales, lower halves bilaterally
Liver	Four fingers below costal margin. Tender	Three fingers below costal margin	Two fingers below costal margin	Not definitely palpable	Questionable due to dyspnea and orthopnea	0	Questionable due to obesity and ascites	Four fingers down
Ascites	0	Marked	Moderate	None	None	0	Moderate	Marked
Peripheral edema	0	++++ General anasarca	++ to +++ extending up to midthigh	None	Minimal, feet and ankles	0	Moderate of feet, ankles and calves. Gradually progressive	++ to +++ lower extremities
Duration of illness	Repeated failures for two years; present episode 1–2 weeks	Progressive dyspnea and edema, 2 weeks. 25 years known RhHD	Rapidly progressive 1 week	Two weeks later	Chronic glomerulonephritis—6 years. Several previous failures	Done when patient fully compensated before present failure	Two months, 11 days treatment before first blood volume was done	Repeated failure since 1920. Five hospitalizations. Progressive failure for past month
V. P.	180	Not done	97	64	Not done	96	Not done	220

TABLE III—*Continued*

	R. S. (2)	L. S.	I. R.	P. D.	C. E.	C. C.	M. B.	S. W.
Age	50	60	34	47	45	44	45	40
Sex	M	F	F	M	F	M	F	F
Diagnosis	RhHD with mitral stenosis and constrictive pericarditis	RhHD with mitral stenosis and insufficiency HCVD	RhHD with mitral stenosis and insufficiency	RhHD with mitral stenosis and insufficiency	RhHD with mitral stenosis and insufficiency	RhHD with mitral stenosis and insufficiency	RhHD with mitral stenosis and insufficiency	HCVD
B. P.	110/80	210/110	120/70	110/70	118/80	126/90	128/110	175/105
Pulse	66	78	130	80	78	50	70	104
Respiration	18	18	32	22	28	20	24	24
Heart	Enlarged, fibrillating, mitral systolic and diastolic murmurs	Enlarged, regular, mitral systolic and diastolic murmurs	Enlarged, fibrillating, mitral systolic and diastolic murmurs	Enlarged, fibrillating, loud systolic mitral murmur	Enlarged, fibrillating, mitral systolic and diastolic murmurs	Enlarged, regular, mitral systolic and diastolic murmurs	Enlarged, fibrillating, mitral systolic and diastolic murmurs	Enlarged, regular. No murmurs
Lungs	Crepitant rales left base. Right lung clear	Crepitant rales both bases	Crepitant rales lower thirds bilaterally	Crepitant rales lower thirds bilaterally	Rales, right base. Left hydrothorax	Rales, left base	Rales left base. Right hydrothorax	Crepitant rales, both bases
Liver	One finger down	Four fingers down	Five fingers down	Three fingers down. Tender	Five fingers down	Four fingers down	Six fingers down	Two fingers down
Ascites	None	None	None	0	0	0	Slight	Questionable, slight
Peripheral edema	Negative	None	None	+++ to knees	++++ to umbilicus	0	Negative	++ feet and ankles
Duration of illness	Four weeks later	Progressive failure for 2 weeks. Several previous failures	Recurrent failures. Present acute episode began 2 days ago	Known RhHD since age 15. Many previous failures. Present episode 2 weeks progressive	Acute rheumatic fever, age 13. First failure 1939. Fairly well until 2 weeks ago. Progressive failure since then	Chronic mild failure 2 years requiring maintenance mercurials. Progressive failure 3 weeks	Chronic failure 4 years. Hospitalization much of the time	One–two months with increasing dyspnea and ankle edema
V. P.	Not done	200	225	Not done	Not done	Not done	Not done	140

TABLE III—*Continued*

	W. N.	H. M.	D. M.	M. K. (*1*)	M. K. (*2*)	M. H.	Al. Gonz.	B. K.
Age	64	44	61	34	34	78	74	70
Sex	M	F	F	F	F	F	M	M
Diagnosis	HCVD	HCVD	Cor. pulmonale. Diffuse pulmonary fibrosis	HCVD	HCVD	ASHD	HCVD	HCVD
B. P.	190/110	250/120	130/90	230/130	200/100	125/60	210/100	180/100
Pulse	80	90	100	84	76	120	120	76
Respiration	30	30	26 Cyanosis of lips and fingers	28	18	30	30	28
Heart	Enlarged, regular. No murmurs	Enlarged, gallop rhythm. No murmurs	Enlarged, regular. No murmurs	Enlarged, regular rhythm. Grade IV mitral systolic murmur	Enlarged, regular rhythm. Grade IV mitral systolic murmur	Enlarged, regular. No murmurs	Enlarged, rapid regular; loud systolic aortic murmur	Enlarged, regular. Grade I mitral systolic murmur
Lungs	Bilateral basal rales	Crepitant rales both bases	Coarse rales, lower halves, bilaterally	Crepitant rales lower thirds bilaterally	Clear	Moist rales lower halves	Crepitant rales both lower thirds	Rales both bases
Liver	One finger down	Three fingers down	0	Five fingers down	Two fingers down	Two fingers down	Two fingers down	Two fingers down
Ascites	Slight amount (autopsy)	Questionable slight	0	0	0	0	0	0
Peripheral edema	++ feet and ankles	++++ lower extremities	Massive extending to nipple line	0	0	+++ to thighs	0	0
Duration of illness	Gradual progressive dyspnea, orthopnea; lower extremities, edema for past 3 months	Weeks, three previous failures in past 5 years	Increasing edema for 2 weeks. Respiratory symptoms minimal	Severe hypertension past 10 years, picked up in pregnancy. Urine clear. Present episode 2–3 weeks	Two weeks later	Increasing failure past month	"Leaky heart" for years. Tachycardia 1 month. Increasing failure for 4 days	Intermittent failure, 6 years. Present episode progressive for past 3 weeks
V. P.	142	150	170	Not done	80	Not done	Not done	Not done

* (*1*) and (*2*) represent clinical findings during failure and after compensation had returned.
† HCVD—Hypertensive Cardiovascular Disease.
‡ ASHD—Arteriosclerotic Heart Disease.
§ RhHD—Rheumatic Heart Disease.

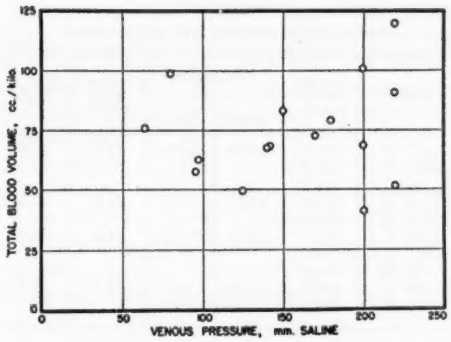

FIG. 2. RELATIONSHIP OF BLOOD VOLUME TO VENOUS PRESSURE, IN CC./KILO

right-sided failure, *b*) primarily left-sided failure, *c*) failure of both sides of heart to a significant degree. We recognize the fact that failure of the right ventricle rarely occurs in the absence of failure of the left, or vice versa. However, the clinical picture is very often determined by marked failure of one side and minimal failure of the other, or marked failure of both sides. Accordingly, the patients have been separated into the above categories. Patients were put in Group A when peripheral edema, liver engorgement, or ascites were marked while dyspnea and pulmonary engorgement played a minor role. They were placed in Group B under circumstances just the reverse of Group A. Group C comprised those patients with significant degrees of failure in the greater and lesser circuits. One patient was in Group A, four in Group B, and seven in Group C.

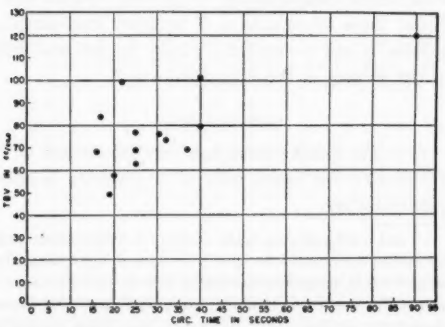

FIG. 3. RELATIONSHIP OF BLOOD VOLUME TO CIRCULATION TIME

It is thus seen that in this group of patients with congestive heart failure and normal blood volume there is not a predominance of left-sided failure. In fact, they comprise only one third of patients designated in Table I. It should be noted that in this group, 11 of the patients were in failure as a result of either arteriosclerotic or hypertensive heart disease and that only one patient had rheumatic heart disease.

All types of failure were found in the group with normal blood volumes. Also to be noted among those in Table I were the four patients where repeat blood volumes were done when compensation had returned. In every case there was a significant rise in blood volume as compensation returned. In three of these patients the total red cell volume and plasma volume rose to an equal degree. The fourth patient showed a much greater increase in plasma volume than total red cell volume.

Table II includes all patients with any elevation of total blood volume, total red cell volume or total plasma volume. There are 15 patients in this group, or 55.5% of the total. There are nine females and six males. This group has been divided into: (A) those whose failure has been caused by old rheumatic valvulitis; (B) others—principally arteriosclerotic heart disease and hypertensive cardiovascular disease. Of the patients in Table II, all had significant involvement of both greater and lesser circulation. No patients with early acute left ventricular failure were available for study during this investigation.

It will immediately be seen that the mean values for the total blood volume and its components in Group B border very closely on normal. It will be noted that four patients in this group were definitely anemic; the anemias being, as far as could be determined, on an azotemic basis. It has been observed that a low total red cell volume may be accompanied by a high plasma volume, presumably a compensatory mechanism through which normal total blood volume is maintained. Therefore, it is not unlikely that these individuals were thrown into the high volume group because of their anemia with compensatory high plasma volumes, rather than as a result of heart failure. Nevertheless, three out of four have normal total blood volumes. Again a rise in blood volume with compensation occurred in one repeat study. In

contrast, the first subgroup, the rheumatics, show strikingly high volumes. The mean value of their blood volume expressed in cc./kilo is 17.9 cc. greater than the mean of the total group in Table I, the total red cell volume and plasma volume being increased to an approximately equal degree. One repeat study done in this group revealed a moderate fall in blood volume when compensation returned, total red cell volume expressed as cc./kilo remaining the same, but plasma volume falling 3.1 cc./kilo. This was the only instance in a total of six repeat determinations in both groups, in which the blood volume did not rise when compensation returned.

As a group, therefore, the rheumatics have a much higher blood volume than those patients without rheumatic heart disease. Nevertheless, one patient with rheumatic heart disease and in failure had an entirely normal blood volume and two patients with nonrheumatic heart disease had very high volumes. In one of these two patients the increase in total blood volume was caused by equal increments of total red cell volume and plasma volume. The other patient had a greater rise in plasma volume than in total red cell volume.

In agreement with Meneely and Kaltreider (3), and as shown by the scatter diagrams in Figures 1 and 2, no correlation was found between either venous pressure or circulation time and blood volume. However, as previously noted, our blood volume data differed markedly from theirs. Using the product moment method, the coefficient of correlation between venous pressure and total blood volume was 0.17. It was not possible to correlate blood volume and venous pressure separately in the right-sided and left-sided failure groups because almost all patients in whom venous pressure was measured had significant failure of both sides of the heart.

The finding of a normal or only minimally increased blood volume in most (18 of 27) patients in frank cardiac decompensation requires a reexamination of some of the current concepts of the pathologic physiology of cardiac decompensation as regards blood volume, particularly in patients with arteriosclerotic heart disease, hypertensive cardiovascular disease, or myocardial infarction, etc.

Of interest are the serum sodium values measured in seven of these patients (Table IV). Three of these seven patients were restudied after complete or partial compensation had occurred. In agreement with previous reports (37) we found no patients with serum sodium concentration elevated beyond the range of normal.[4] In those with repeat studies after compensation, no significant fall in serum sodium values (average of 3 meq.) was found. Total exchangeable body sodium determinations were done in four of these patients using Na^{24} and invariably high values were found in failure. Sodium has been found to equilibrate rapidly between plasma and extracellular fluid (39, 40). Any increase in sodium level of the body results in concomitant expansion of the sodium (approximately extracellular) space rather than a rise in sodium concentration. Contrary to the suggestion of Warren and Stead (41) the plasma volume did not rise as the interstitial space expanded, nor vice versa, in five out of six cases studied during and after failure. In fact, just the opposite occurred. As compensation returned, with edema disappearing, the plasma volume rose. From these observations it appears that plasma volume is not controlled directly by rise and fall of the volume of the interstitial space.

TABLE IV

Serum sodium and total body sodium values

Patient	Serum sodium (*meq.*)	Total sodium		Sodium space (*liters*)	% Body weight
		(*meq.*)	(*gm.*)		
1 C. P.	145.7	4,939	113.6	33.9	57.0
2 C. P.	143.0				
A. G.	145.5				
1 M. K.	143.0	3,147	72.4	22.0	42.5
2 M. K.	140.0				
1 C. R.	147.8	5,084	116.9	34.4	50.0
2 C. R.	142.0				
H. M.	147.0				
M. G.	146.0	3,720	85.6	25.5	41.5
C. W.	148.0				

CONCLUSIONS

(*1*) The blood volume has been determined with P^{32} labeled red blood cells in 27 patients in congestive failure.

[4] Total exchangeable body sodium determinations and serum Na concentrations have been done in approximately 30 patients in congestive failure by Warner and his associates (35) in this laboratory using Na^{24} and the flame photometer method (38). They also have found markedly elevated total exchangeable body sodium levels, and serum sodium concentrations which vary from normal to low.

(*2*) Twelve of 27 patients had normal blood volume, total red cell volume and plasma volume. In 11 of these patients the failure was due to arteriosclerotic heart disease or hypertensive cardiovascular disease; in one, a result of old rheumatic valvulitis.

(*3*) Fifteen of the 27 patients had either an elevated blood volume, total red cell volume, or plasma volume. These 15 patients were divided into two groups: (A) those with rheumatic valvulitis, seven; and (B) those with hypertensive cardiovascular disease or arteriosclerotic heart disease, eight. In Group A the total blood volumes were markedly increased, total red cell volume and plasma volume rising in equal degrees; in Group B, only slightly increased, the degree of rise in total red cell volume and plasma volume varying markedly from patient to patient.

(*4*) In five of six instances studied, the blood volume increased when compensation occurred. In the sixth patient, blood volume decreased with return of compensation.

(*5*) Serum sodium concentration did not change markedly during heart failure, but total body sodium rose to a marked degree.

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STUDIES IN FAT METABOLISM. I. THE COLORIMETRIC DETERMINATION OF KETONE BODIES IN BIOLOGICAL FLUIDS[1]

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The lack of a simple and accurate method for the quantitative determination of ketone bodies in biological fluids accounts in large measure for the relatively small amount of data in the literature relating to ketone metabolism. Most of the methods which have so far been described have required the conversion of β-hydroxybutyric and acetoacetic acids to acetone, the distillation of the acetone so formed, and the quantitative determination of the acetone in the distillate by gravimetric, titrimetric, or colorimetric procedures. The most serious limitation of these procedures lay in the likelihood of loss of acetone during distillation. The necessity for the use of a reflux condenser in the process of conversion of acetoacetic acid and β-hydroxybutyric acid to acetone also constituted a source of possible loss of acetone. The percentage loss, when one was dealing with small quantities of acetone, could be of considerable magnitude.

The method to be described eliminates distillation and refluxing. It makes use of the colorimetric determination of acetone phenylhydrazone described by Greenberg and Lester (1). The following difficulties were encountered in the method as described by these authors:

1. Acetoacetate in the presence of acid dichromate instead of being entirely decarboxylated to acetone is partially and unpredictably converted to compounds other than acetone.

2. As the result of inadequate time of refluxing and possibly to too concentrated a dichromate solution, predictable recovery of β-hydroxybutyrate was not possible.

[1] This work is supported by grants from the Commercial Solvents Corporation and from the Armour Laboratories.

[2] Damon Runyon Clinical Research Fellow, 1949 to present.

EQUIPMENT AND REAGENTS

Equipment

1. Narrow neck, screw top, heavy rim, 4 ounce bottles.[3]
2. Heat resistant, plastic screw caps to fit these bottles.[3]
3. Washers for the screw caps cut from Goodrich Ameripol D rubber sheets.[4] The washers were treated by boiling for 15 minutes in approximately 2 normal sodium hydroxide, washed in running tap water, rinsed in distilled water, soaked in carbon tetrachloride for about 30 minutes, and shaken in the dinitrophenylhydrazine solution for 10 minutes before their initial use. This treatment removed interfering materials from the synthetic rubber. Other rubber products, both natural and synthetic, were tried but were not satisfactory.

Reagents

1. Barium hydroxide, 0.15 *N*.[5]
2. Zinc sulfate, $ZnSO_4 \cdot 7H_2O$, 2.5 gm./100 cc. of water.[5]
3. Sulfuric acid, 13.6 *N*.
4. Potassium dichromate, 0.4 gm./100 cc. of water.
5. Sodium sulfite, 5.0 gm./100 cc. of water.
6. 2:4 dinitrophenylhydrazine, 0.1 gm. in 100 cc. of 2.0 *N* HCl (filter before use).
7. Carbon tetrachloride (reagent grade).
8. Sodium hydroxide, 10.5 *N*.

DETERMINATION OF KETONES IN WHOLE BLOOD

Precipitation of blood protein—(a modification of the method of Weichselbaum and Somogyi [2])

Add, with shaking, 5 cc. of unclotted blood (collected in a heparin or Wintrobe oxalate tube) to 15 cc. of distilled water in a small flask. Allow five minutes for complete hemolysis.

[3] Owens-Illinois glass bottles No. A-4254 fitted with 20 mm. black plastic screw caps have proven satisfactory.

[4] Acknowledgment is made to the B. F. Goodrich Company for supplies of Ameripol D synthetic rubber.

[5] The concentration of these reagents must be so adjusted that 5.0 cc. of the zinc sulfate solution, diluted to a volume of 25 cc. will require 5.0 cc. of the barium hydroxide solution to produce a permanent pink color, using phenolphthalein as an indicator.

Then add slowly 15 cc. of the barium hydroxide solution (with shaking) followed by 15 cc. of the zinc sulfate solution. Allow at least 10 minutes for complete precipitation of the blood protein. Filter through a good grade paper such as Whatman No. 40.

Hydrolysis and oxidation of keto acids

To each of two bottles, add 5 cc. of the whole blood filtrate and 1 cc. of 13.6 *N* sulfuric acid. Place caps on tightly (with pliers to avoid any possible loss of acetone) and autoclave for 10 minutes at 15 to 20 lbs. pressure. This decarboxylates all of the acetoacetic acid to acetone, and does not affect the β-hydroxybutyric acid.

After removal from the autoclave and cooling to room temperature, add 1 cc. of 0.4% potassium dichromate solution. One bottle is used directly for the determination of preformed acetone plus acetone derived from acetoacetic acid. Oxidation and hydrolysis of β-hydroxybutyric acid is carried out in the other by autoclaving for 30 minutes (bottle tightly sealed). Under these conditions a quantitative conversion of β-hydroxybutyric acid to acetone is achieved.[6]

Quantitative determination of acetone

To each bottle add 1 cc. of 5% sodium sulfite,[7] replace the caps and mix thoroughly. The sodium sulfite reduces the free dichromate which otherwise would oxidize both the hydrazine and the hydrazone. Add 5 cc. of the acid dinitrophenylhydrazine solution and 5.0 cc. of carbon tetrachloride. Recap the bottles and shake for 10 minutes at a rate of approximately 260–275 times per minute (any Kahn shaker is satisfactory). Add 3.0 cc. of 10.5 *N* sodium hydroxide, shake for five minutes, and pour the entire contents of each bottle into a test tube. Remove the supernatant aqueous layer by suction, and compare the color of the carbon tetrachloride fraction with the appropriate standard.

The color of the standards is obtained from the reaction of 5.0 cc. of an aqueous acetone solution (0.1 cc./liter or 7.92 mg./100 cc.); the color of the blanks, from the reaction of 5 cc. of water. In each instance the material is treated in the same manner as the whole blood.

We recommend for acetone concentrations in the blood ranging from 2 to 29 mg./100 cc., a wave length of 420 millimicrons; for a range from 0 to 2 mg./100 cc., a wave length of 375 millimicrons. The use of a Klett-Somerson or comparable colorimeter, employing a blue filter, gives satisfactory results.

Since Beer's Law holds, in a range of 0 to 14 mg./100 cc., the concentration of ketones in the blood (in terms of acetone equivalents, mg./100 cc.) will equal 7.92·Du/Ds (Figure 1).

[6] Standard β-hydroxybutyrate obtained from British Drug Houses, Ltd.

[7] Prepare a fresh solution every week.

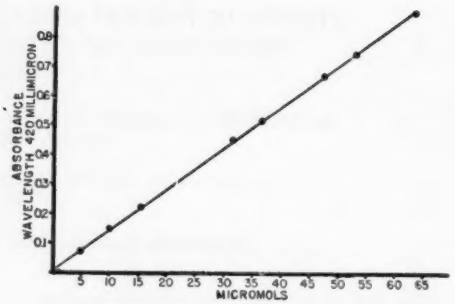

FIG. 1. RECOVERY OF ACETONE

Standard curve.

Procedure for determination of urine ketones

Urine ketones can be determined in the same way. Protein precipitation is unnecessary unless proteinuria is present. A 1:10 dilution has been found to be satisfactory for normal urines.

RESULTS

The recovery of acetoacetic acid and β-hydroxybutyric acid in aqueous solution or added to blood is shown in Table I.

In the present method, in the absence of dichromate, 100% conversion of acetoacetate to ace-

TABLE I

Recoveries of acetoacetic and β-hydroxybutyric acids
(expressed as acetone)

Solvent	Amount added (*mg./100 cc.*)	Amount recovered (*mg./100 cc.*)	Recovery (*per cent*)	
Water	6.34 4.75 3.17 1.56	6.34 4.70 3.20 1.58	100 99 101 101	Acetone in H_2O
	6.34	6.34	100	Acetone in H_2O, treated with $ZnSO_4$ and $Ba(OH)_2$
	4.58	4.53	99	Ethyl acetoacetate from H_2O
	4.58	4.50	98.5	Ethyl acetoacetate from H_2O treated with $ZnSO_4$ and $Ba(OH)_2$
	6.82	6.82	100	β-hydroxybutyric acid from H_2O
	6.82	6.74	98.8	β-hydroxybutyric acid from H_2O treated with $ZnSO_4$ and $Ba(OH)_2$
Blood	0	0.8		Acetoacetic acid
	0	0.25		β-hydroxybutyric acid
	4.58	5.30	98.5	Ethyl acetoacetate
	6.82	6.78	99.4	β-hydroxybutyric acid

All determinations were done in quadruplicate with a variation of less than ± 1%.

TABLE II

Results from analysis of blood of patients

Patient	Acetone and acetoacetic acid (*mg./100 cc.*)	β-hydroxybutyric acid (*mg./100 cc.*)	Total ketones (*mg./100 cc.*)	Diagnosis
D. E. P.	0.31	0.53	0.84	Cirrhosis and nephritis
R. O. M.	0.03	0.35	0.38	Hepatitis, acute
F. E. R.	0.07	0.52	0.59	Hepatitis, chronic
B. R. A.	0.38	0.45	0.83	Jaundice, obstructive
T. U. C.	0.12	0.40	0.52	Cirrhosis
N. I. C.	0.34	0.49	0.83	Congestive failure
L. A. R.	0.33	0.26	0.59	Cirrhosis
S. P. I.	0.20	0.52	0.72	Hypogonadism
R. O. S. 24 hr. fasting	3.5	7.5	11.0	Diabetic
48 hr. fasting	14.5	19.6	34.1	Diabetic
72 hr. fasting	28.5	29.5	58.0	Diabetic

tone is obtained. In the presence of dichromate, as in the method of Greenberg and Lester (1), only a 40-60% recovery results. Recovery of β-hydroxybutyrate by our method was consistently $100 \pm 1\%$. $ZnSO_4$ and $Ba(OH)_2$ did not alter the recoveries.

α-Ketoglutaric acid, lactic acid, and pyruvic acid in concentrations of 1 to 50 mg./100 cc. did not interfere with quantitative recovery of acetone, acetoacetate, or β-hydroxybutyrate.

Blood sugars in excess of 200 mg./100 cc. result in high ketone values. Appropriate dilutions must be made for such bloods.

In Table II are shown the fasting blood ketones obtained in a group of patients, including three consecutive values in a fasting diabetic over a 48 hour period.

SUMMARY

A quantitative colorimetric method for ketones in biological fluids is presented. It eliminates the need for distillation and refluxing.

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STUDIES IN FAT METABOLISM. II. EVALUATION OF THE EFFECT OF TESTOSTERONE PROPIONATE UPON KETONE, CARBOHYDRATE, AND PROTEIN METABOLISM IN A PATIENT WITH DIABETES MELLITUS COMPLICATED BY THYROTOXICOSIS [1, 2]

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(Submitted for publication June 18, 1951; accepted August 27, 1951)

In 1937, Kochakian observed that testosterone compounds, when administered to castrate dogs, resulted in nitrogen retention (1). This coincided with the acquisition of a proportional amount of protein tissue in such animals. An identical effect in man was demonstrated by Kenyon and his co-workers (2), and subsequently by others including Albright, Brown, Butler, Howard, Talbot, Wilkins, and their respective co-workers.

Albright noted that the administration of testosterone to a fat man on a low caloric intake resulted in diminished nitrogen in the urine and cessation of weight loss (3) (Figure 1).[5] The explanation of this phenomenon is not hard to find. The energy equivalent of protein tissue (which is about 80% water) is approximately 0.8 calorie per gm.; that of fat tissue (which contains only 10% water) is 8 to 9 calories. Therefore, 1 gm. of fat tissue yields approximately 10 times the number of calories obtainable from 1 gm. of protein tissue. An individual who spares protein tissue and who oxidizes fat tissue in an amount calorically equivalent to such protein tissue will consume only one-tenth the weight of tissue in the course of such oxidation. In other words, when one burns fat and builds

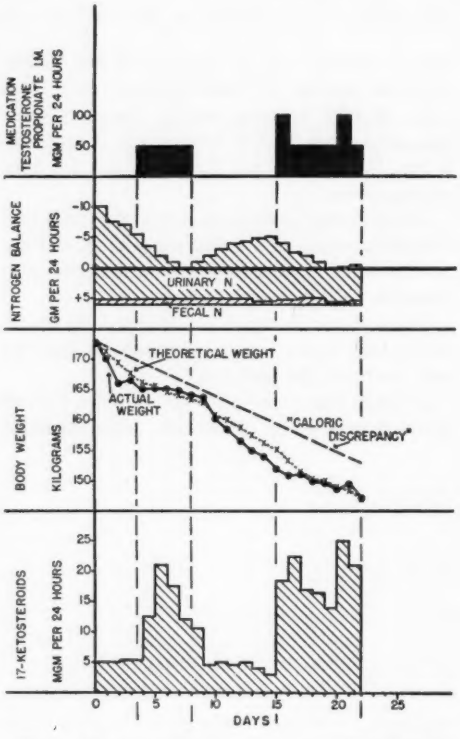

FIG. 1. PROTEIN-SPARING ACTION OF TESTOSTERONE PROPIONATE IN AN OBESE INDIVIDUAL ON A LOW CALORIE INTAKE

(Modified from Albright.[5])

[1] This work was supported in part by grants from the Schering Corporation; Research Division of the Bureau of Medicine and Surgery, U. S. Navy (BuMed No. 007046) and from the Office of Naval Research, under a contract between the latter and the University of California.

[2] This paper was presented in part at a joint meeting of the American Diabetes Association and the Association for the Study of Internal Secretions at Atlantic City, New Jersey, on June 4, 1949.

[3] Schering Research Fellow in Endocrinology, 1948–1949; Damon Runyon Clinical Research Fellow, 1949–1951.

[4] HM$_1$, USN.

[5] Used with the permission of Dr. Fuller Albright and the Josiah Macy, Jr. Foundation.

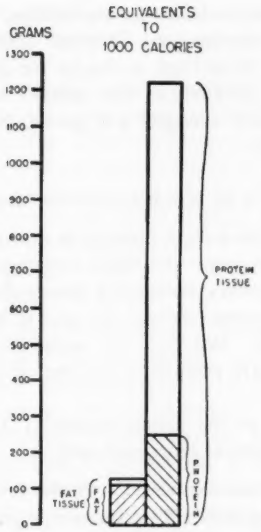

FIG. 2. CALORIC EQUIVALENTS OF FAT AND PROTEIN TISSUE

It is apparent that the combustion of 1 gm. of fat tissue can spare more than 10 times that mass of protein tissue.

protein, he can lay down approximately 10 gm. of protein tissue for every gram of fat tissue which is oxidized. This is depicted diagrammatically in Figure 2.

An agent such as testosterone, therefore, which has a major protein anabolic effect, and which can exert a protein sparing action in individuals on a low calorie intake might produce such an effect by accelerating the rate of fat oxidation. The present investigation was designed to evaluate this possibility.

EXPERIMENTAL CONDITIONS

The patient selected for the study was a 27 year old male with severe diabetes, complicated by severe thyrotoxicosis (his basal metabolic rate was consistently in excess of plus 80%).[6]

The nature of the metabolic abnormalities made the production of any desired degree of ketonemia, ketonuria, hyperglycemia, and glycosuria a simple matter. Throughout the study, his dietary intake was in excess of 3900 calories and consisted of 128 gm. of protein, 264 gm. of fat, and 259 gm. of carbohydrate. The diet was chemically constant throughout. Its composition was checked at regular intervals by chemical analyses of homogenized aliquots. Diet and crystalline insulin (155 units daily) were administered to him in five portions between the hours of 10:00 a.m. and 10:00 p.m. On this diet and on a program of constant activity, he was in weight equilibrium, and his urinary nitrogen averaged 19 gm. daily for a period of more than two weeks prior to the administration of any therapeutic agent other than insulin.

At 8:00 each morning (10 hours after the patient had received his last food and last insulin), fasting blood specimens were obtained for sugar and ketones. Twenty-four hour urine collections were divided into three component portions: the first, from 6:00 to 8:00 a.m.; the second, from 8:00 to 10:00 a.m.; and the third, from 10:00 a.m. to 6:00 a.m. the following morning. Sugar, total ketone, nitrogen, sodium, phosphorus, potassium, and other determinations were carried out in these specimens.

Respiratory quotient determinations were obtained between the hours of 8:00 and 9:00 a.m. daily. All respiratory quotient data are corrected for urinary sugar and urinary nitrogen.

METHODS

The urinary glucose was determined by Benedict's method (4), and the blood sugars by Nelson's modification of the Somogyi procedure (5). The blood and urinary ketones were quantitated by the method of Barnes and Wick (6) (this study was carried out prior to the development of the method of Michaels and associates, Paper I of this series), and the urinary nitrogens by the Kirk modification of the Kjeldahl procedure (7). Respiratory quotients were determined with the use of a closed circulating system in which the oxygen was measured by a precision wet test meter, and the carbon dioxide was absorbed in a solution of sodium hydroxide. The carbonate formed was quantitated in a Van Slyke manometric blood gas apparatus (8). As noted above, all respiratory quotient values were corrected for urinary sugar and for urinary nitrogen. The total amount of urinary ketones was not of sufficient magnitude to warrant the use of a correction factor.

RESULTS OF METABOLIC STUDY

In Figure 3 are shown the data dealing with urinary sugar, ketones, and nitrogen, fasting blood sugar and ketones, respiratory quotient, basal oxygen consumption, and daily weight, obtained over a period of 25 days in the man above described. Because of the length of the study, only four days of control are shown. The patient, however, had been in a state of essential equilibrium for a period of two weeks prior to the administra-

[6] This patient was transferred to our service specifically for this study through the kindness of Dr. Gordon E. Hein and Dr. Felix Kolb of the Veterans Administration Hospital, San Francisco.

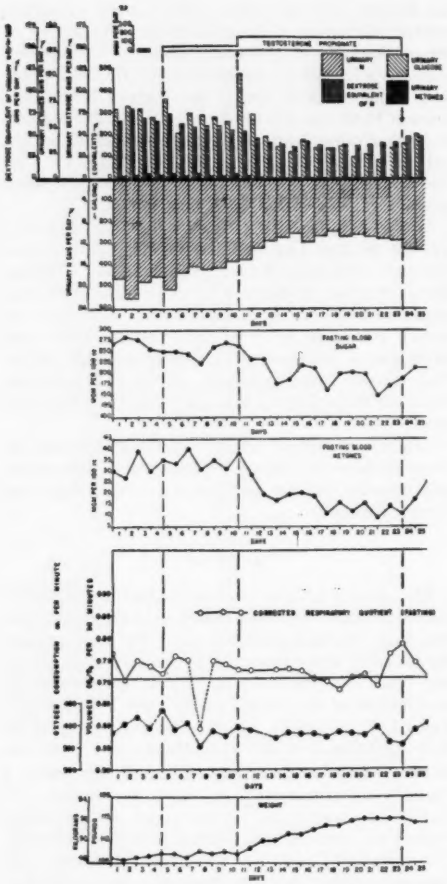

FIG. 3. THE EFFECTS OF TESTOSTERONE PROPIONATE UPON BLOOD AND URINARY SUGAR AND KETONES, URINARY NITROGEN, RESPIRATORY QUOTIENT, BASAL OXYGEN CONSUMPTION, AND BODY WEIGHT IN PATIENT WEL

tion of testosterone. In chronological sequence, the observations are as follows:

Pre-testosterone

During this time the patient excreted approximately 75 gm. of glucose, 5 gm. of urinary ketones, and 19 gm. of urinary nitrogen, daily.

His fasting blood sugar averaged 275 mg./100 cc., and his fasting blood ketones, 35 mg./100 cc. *Non-protein* respiratory quotients, as one would expect, averaged about 0.73, a value compatible with almost exclusive fat combustion. (The respiratory quotient was obtained after complete abstinence from food or insulin for a period of more than 10 hours.) The patient's weight during this time averaged 106 pounds with minor fluctuations.

Response to 50 mg. of testosterone propionate[7,8]

Except for a slight decrease in urinary nitrogen and urinary sugar and slight weight gain, no significant changes were noted during the six days that the patient received this dose of testosterone propionate. We have no explanation for the single bizarre respiratory quotient of 0.59.

Response to the administration of 150 mg. of testosterone propionate daily

In previous experience with the use of testosterone in normal individuals and in patients with various metabolic abnormalities, we have felt that 50 mg. of testosterone propionate daily produced almost as great metabolic effects as would larger dosage. In this man the following changes immediately followed the administration of 150 mg. of testosterone propionate daily, whereas as noted above, the smaller dose produced minimal changes in this portion of the metabolic pattern:

1. *Urinary sugar,* after an increase to approximately 125 gm. on the first day of the large dose of testosterone, fell steadily to values approximating 25 gm. of glucose daily.

2. *Urinary nitrogen* decreased to an average level of 10 gm. daily, that is, to approximately half the amount excreted prior to the administration of testosterone.

3. *Fasting blood sugar* fell as low as 150 mg./100 cc.

4. *Fasting blood ketones* fell from their previous high levels to average values of 10 mg./100 cc.

5. *Urinary ketones* reached negligible levels.

[7] Grateful acknowledgment is made to Dr. Edward Henderson, Research Director, Schering Corporation, for generous supplies of testosterone propionate (Oreton) used in this study.

[8] Testosterone propionate was administered intramuscularly. During the time of administration of 150 mg. daily, 50 mg. were given at 3 and 6 a.m., and 25 mg. at 10:00 a.m. and 10:00 p.m.

6. The corrected (non-protein) *respiratory quotient* fell to values which on some occasions were below 0.71.

7. *Basal oxygen consumption* did not change significantly. It was, however, subject to less daily fluctuation. This could be accounted for by the greatly improved clinical status of the patient.

8. *Weight gain* of 10 pounds occurred during the 13 days that the patient received 150 mg. of testosterone propionate daily.

Clinical status of the patient

Prior to the administration of testosterone, he had been very irritable in the early morning. This perhaps was attributable in part to the lack of adequate carbohydrate combustion and to the moderate ketosis. Within the three days following the beginning of testosterone administration, a most impressive change occurred in the clinical picture. He awakened spontaneously, was thoroughly cooperative, and, although still quite hyperkinetic (as one would expect in any individual with a basal metabolic rate in excess of plus 80), became a model patient. Prior to this, quite close supervision had been necessary to make sure that diet, activity, and medication were constant.

Additional studies

In addition to the studies just noted, determinations of urinary phosphorus, potassium, sodium, and other constituents were carried out during the first 25 days and also during periods of withdrawal and resumption of testosterone administration and administration of other hormonal agents. These studies will be described elsewhere in conjunction with similar studies in patients with uncomplicated thyrotoxicosis and uncomplicated diabetes. Suffice it to say for the present, that when testosterone was withdrawn prompt "rebound" occurred in terms of increased urinary nitrogen, sugar, ketones, and rapid deterioration in the clinical status of the patient.

DISCUSSION

The administration of testosterone propionate in adequate dosage to a patient with severe diabetes mellitus, associated with severe thyrotoxicosis, produced major metabolic changes which may be summarized as follows:

1. Little or no decrease in basal oxygen requirement.

2. Inhibition of protein catabolism, as manifested by decrease in nitrogen excretion, to approximately two-thirds of the previous amount.

3. A rapid fall in fasting blood sugar, and a decrease in urinary sugar excretion to less than one-half the previous amount.

4. A fall in fasting blood ketones to less than 25% of the previous values, and an equivalent fall in urinary ketone excretion.

5. Rapid weight gain roughly proportional to the decrease in nitrogen excretion.

6. Fasting, non-protein, respiratory quotient values compatible with nearly exclusive fat combustion.

Interpretation of the preceding observations is not too simple. A portion of the interpretations must at the present time be speculative.

Factual interpretations

1. The energy requirement in this patient was not diminished.

2. The fall in fasting blood sugar and in urinary sugar appears to be attributable largely or entirely to decreased protein catabolism. Inspection of Figure 1 reveals that the decrease in urinary sugar and the fall in dextrose equivalent of nitrogen (D/N = 3.65) are reasonably parallel.

3. Consequently there was little or no increased energy derived from glucose inasmuch as the changes in blood sugar could be accounted for almost entirely on the basis of decreased carbohydrate formation rather than increased carbohydrate utilization.

4. Since the patient actually gained weight, had no increased calories available from carbohydrate, diminished calories available from protein, and had no decrease in energy requirement, it appears that he must of necessity have had an increased catabolism of fat.

5. Despite such increased fat catabolism, the blood and urinary ketones fell to an extreme degree.

Speculative interpretations

The observation of decreased blood and urinary ketones despite an increase in fat catabolism suggests that testosterone propionate in this patient may have:

1. Decreased the rate of ketone formation from fatty acids, or

2. Increased the rate of ketone utilization.

Other studies will attempt to elucidate the mechanisms involved.

SUMMARY

A patient with severe diabetes mellitus associated with severe thyrotoxicosis, on a chemically constant dietary intake and constant insulin therapy, during the administration of 150 mg. of testosterone propionate daily manifested a fall in urinary sugar, nitrogen, and ketones of quite marked degree.

It is suggested that under these experimental conditions, the changes observed may have been due in part at least to a direct effect of testosterone upon fat or ketone metabolism.

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STUDIES IN FAT METABOLISM. III. THE EFFECT OF ACTH, OF CORTISONE, AND OF OTHER STEROID COMPOUNDS UPON FASTING-INDUCED HYPERKETONEMIA AND KETONURIA [1, 2, 3]

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(Submitted for publication June 18, 1951; accepted August 27, 1951)

As previously described (1), testosterone propionate appears to have a direct or indirect effect upon fat metabolism. The present study was initiated for the purpose of evaluating further the effect of testosterone and of other hormonal entities upon fat metabolism.

A standard fasting technique was devised as a reasonably simple method of inducing hyperketonemia. Initially, patients were fasted for two and one-half days, and subsequently for three and one-half days. As expected, almost all individuals had a progressive rise in blood and urinary ketone bodies during the period of the fast. Each individual was used as his own control, that is to say one or more control fasts were carried out during which time no treatment was administered. Subsequently, one or more identical fasts were carried out during which time the patient was treated with specific hormonal agents.

METHODS

In addition to blood and urinary ketones, urinary nitrogen, sodium, potassium, and 17-ketosteroids have been determined, as well as blood sugar, serum phosphorus, and circulating eosinophil levels. Urinary nitrogen has been determined by the Kirk modification of the Kjeldahl procedure (2). Urinary sodium and potassium have been quantitated using the Beckman flame photometer. Blood and urinary ketones have been determined by the method of Michaels and associates (3); serum phosphorus by the method of Fiske and SubbaRow (4); blood sugar by the method of Giragossintz, Davidson and Kirk (5); and circulating eosoinophils by the method of Randolph (6).

RESULTS

A total of more than 150 fasts in more than 50 patients have been carried out to date. As noted above, the procedure initially consisted of a two day fast. In Figure 1 is shown a summary of some of the findings in the first patient in whom the fasting procedure was instituted, a 45 year old male with relatively mild diabetes mellitus. This patient, at the time that the studies were first begun, had received no insulin at any time. He had an initial fasting blood sugar which approached 250 mg./100 cc., and a fasting blood ketone level of approximately 10 mg./100 cc. (normal value less than 1 mg./100 cc.). During the first two and one-half day fast, the patient, after an initial fall in blood ketones, had a progressive rise to a level in excess of 30 mg./100 cc. Also during the first 36 hours, there was a fall in blood sugar from his original level to values of less than 150 mg./100 cc.

The second fast was carried out a week later. It is of interest that following the initial fast, this man's fasting blood sugar values remained below 200 mg./100 cc. Testosterone propionate [4] was administered in a dosage of 100 mg. daily beginning four days before fast No. 2. During this fast, the blood sugar values fell to completely normal levels (normal range for this method varies from 60 to 100 mg./100 cc.). Initial fasting blood ketones were in a normal range and rose progres-

[1] This work is supported in part by grants from the Pharmaceutical and Research Divisions, Armour and Company, and from the Office of Naval Research.

[2] A portion of this work was carried out on the wards of the hospital of the University of California Medical School, San Francisco.

[3] A portion of this work appears in the Proceedings of the Second Clinical ACTH Conference, Volume I—Research, John R. Mote, Ed., Blakiston Co., 1951, pages 308–317.

[4] Grateful acknowledgment is made to Dr. Edward Henderson of the Schering Corporation for supplies of testosterone propionate.

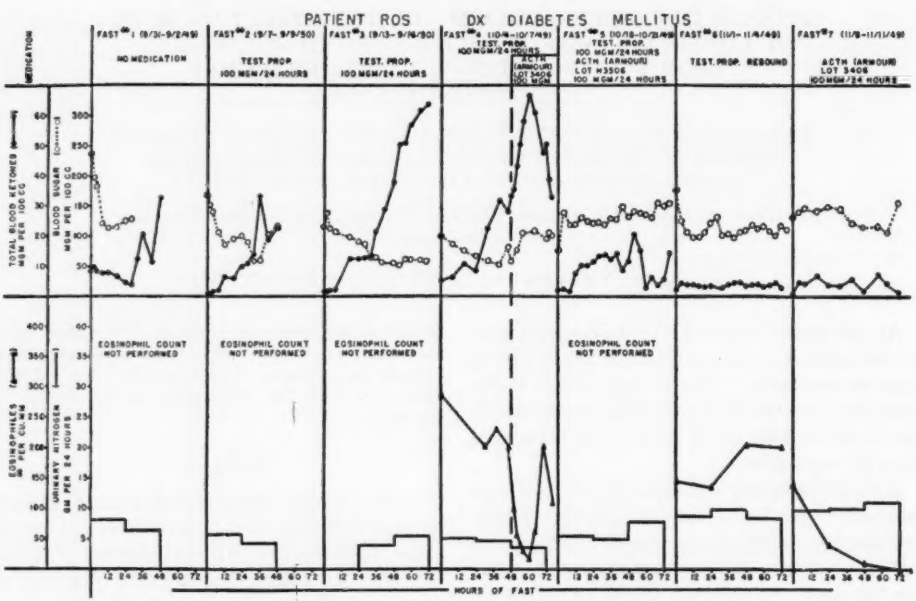
PATIENT ROS DX DIABETES MELLITUS
MEDICATION
FAST** 1 (9/24-9/29/49)
NO MEDICATION
FAST** 2 (9/7-9/9/50)
TEST. PROP 100 MGM/24 HOURS
FAST** 3 (9/13-9/16/50)
TEST. PROP. 100 MGM/24 HOURS
FAST** 4 (10/18-10/21/49)
TEST. PROP. 100 MGM/24 HOURS
ACTH (ARMOUR) LOT W3506 100 MGM/24 HOURS
FAST** 5 (10/18-10/21/49)
TEST. PROP. 100 MGM/24 HOURS
ACTH (ARMOUR) LOT W3506 100 MGM/24 HOURS
FAST** 6 (11/1-11/4/49)
TEST. PROP. REBOUND
FAST** 7 (11/8-11/11/49)
ACTH (ARMOUR) LOT 3406 100 MGM/24 HOURS
TOTAL BLOOD KETONES MM. PER 100 CC.
BLOOD SUGAR MGM PER 100 CC.
EOSINOPHILES PER CU. MM.
URINARY NITROGEN GM PER 24 HOURS
EOSINOPHIL COUNT NOT PERFORMED
HOURS OF FAST

FIG. 1. EFFECT OF TESTOSTERONE PROPIONATE AND OF ACTH UPON FASTING-INDUCED HYPERKETONEMIA IN A PATIENT WITH MILD DIABETES MELLITUS

It is apparent that: a. Testosterone propionate did not significantly modify the fasting-induced hyperketonemia. b. ACTH depressed to a marked degree the fasting-induced hyperketonemia. c. Fasting-induced hyperketonemia was greatly depressed during the period of "testosterone rebound."

sively during the two and one-half days of the fast. The urinary nitrogen excretion was significantly less during this fast as compared with fast No. 1. Testosterone propionate was administered continuously in the dosage above noted over the next several weeks (until the completion of fast No. 5).

Fast No. 3 was carried out over a period of 72 hours. At the end of this period, the fasting blood ketones exceeded 60 mg./100 cc.

During the last 24 hours of fast No. 4, the patient received 25 mg. of ACTH [5] intramuscularly every two hours for four doses(first dose at 48th hour of fast). The blood ketones which had risen precipitously, as in fast No. 3, fell from a level of 68 mg. % to 28 mg. % during the last 18 hours of the fast. No increase in urinary nitrogen occurred despite the ACTH administration.

During fast No. 5, the patient continued to receive testosterone and also received ACTH—25 mg. every six hours during the period 10/18 to 10/21. *The blood ketones at no time exceeded 20 mg./100 cc.* Urine nitrogen excretion was higher than during the fast on testosterone alone, and the blood sugars rose to levels approaching 150 mg./100 cc.

On October 28, testosterone was discontinued; and fast No. 6 was performed during the period of November 1st to 4th. During this period of "testosterone rebound" (that is during the period of increased protein catabolism which always occurs when testosterone is discontinued), the blood ketone levels remained below 5 mg./100 cc. throughout the entire fast; the urine nitrogens remained high; and the blood sugar levels averaged 100 mg./100 cc. There was no fall in circulating eosinophils.

The final fast (No. 7) was performed one week later during which time the patient again received 25 mg. of ACTH every six hours throughout the

[5] Grateful acknowledgment is made to Dr. John R. Mote of the Armour Laboratories for supplies of ACTH.

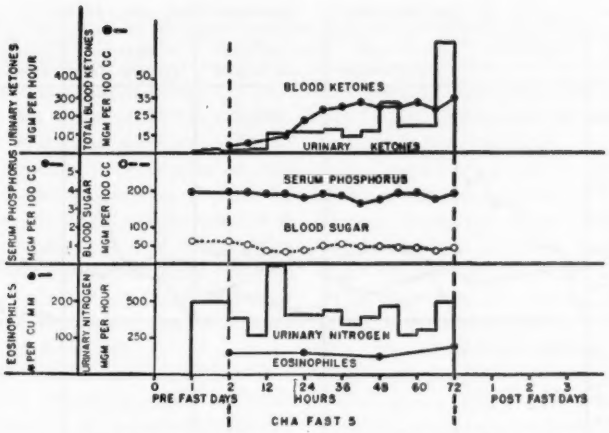

FIG. 2. CONTROL FAST IN A "NORMAL" OBESE MALE

period November 8th to November 11th. No other medication was administered. The blood ketone values during this fast were comparable to those during the period of "testosterone rebound." The blood sugars and the urinary nitrogens were slightly higher; and the circulating eosinophils fell to 0, indicating an adequate adrenal response to the administered ACTH.

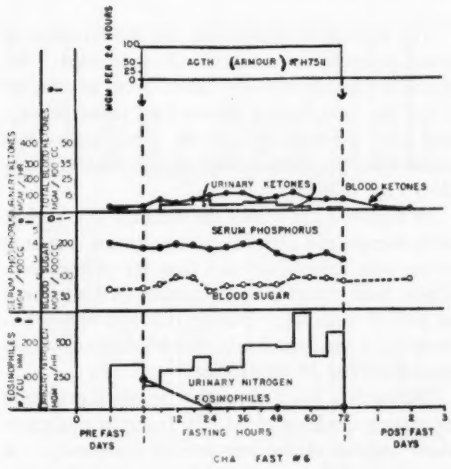

FIG. 3. FAST WITH ACTH IN THE SAME PATIENT SHOWN IN FIGURE 2

The marked decrease in hyperketonemia and ketonuria is apparent.

A large group of patients without evidence of any endocrine abnormality has been studied in the manner indicated above. In Figures 2 and 3 are shown the values obtained during a control fast and during a fast with ACTH treatment (25 mg. every six hours) in a man with simple exogenous obesity. During the control fast the blood ketones rose to levels approximating 35 mg./100 cc. An identical fast carried out in conjunction with ACTH administration resulted in final blood ketone values which were less than 10 mg./100 cc. The urinary ketone excretion was proportional to the blood levels. Blood sugar levels were significantly lower during the control fast than during ACTH administration. Urinary nitrogen values tended to be relatively constant during the control fast, and to rise during the fast with ACTH. An excellent eosinophil response was observed during the "ACTH fast."

In Figure 4 are shown serial studies in a young male suffering from rheumatoid arthritis. During control fast No. 1 (August 29–September 1), his blood ketones rose to values in excess of 40 mg./100 cc., and his blood sugars fell.

ACTH in the usual dosage (100 mg. daily) was begun on September 12th. During fast No. 2 (September 12–15), the fasting blood ketones did not exceed 15 mg./100 cc. A third fast carried out two weeks later (during which time the patient had received ACTH continuously) showed a maximal blood ketone value only slightly in ex-

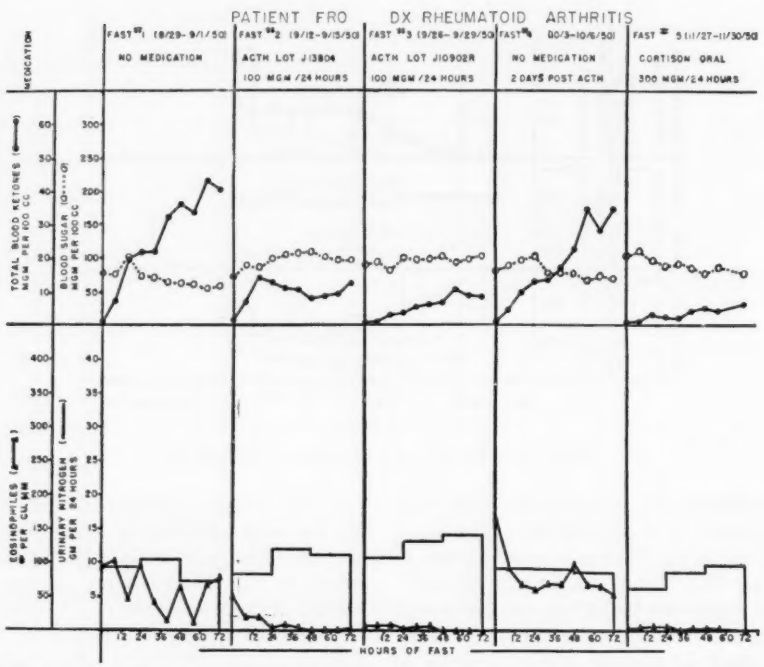

FIG. 4. SERIAL FASTING STUDIES IN A YOUNG MALE PATIENT WITH RHEUMATOID ARTHRITIS
Both ACTH and cortisone greatly diminish the fasting-induced hyperketonemia.

cess of 10 mg./100 cc., and an average level much below this.

Fast No. 4 carried out two days after discontinuance of ACTH resulted in a rise of blood ketones to approximately 35 mg./100 cc. on the third day.

Fast No. 5 carried out six weeks later, during which time oral cortisone [6] was administered continuously in a dosage of 300 mg./24 hours, resulted in maximal blood ketone levels well below 10 mg./100 cc.

Fasting alone was associated with a tendency to fall in circulating eosinophils, but the magnitude of this fall was not comparable with that resulting from ACTH and cortisone. Urinary nitrogen excretion and blood sugar levels were significantly higher during the fasts associated with ACTH administration.

[6] Grateful acknowledgment is made to Dr. Augustus Gibson of Merck & Company for supplies of cortisone and Compound A.

The findings in this patient are characteristic of those observed in more than 20 individuals with rheumatoid arthritis who have been so studied (with the exception of patient Lew noted below), and also characteristic of the other individuals studied excepting those with specific endocrinopathies (see below).

In Figure 5 are shown the findings in a patient with rheumatoid arthritis, whose ketone response is the only exceptional one thus far encountered. There were other bizarre features in his pattern of ACTH response. During fast No. 1, this patient had a maximal rise in blood ketones to values approximating 35 mg./100 cc.

During fast No. 2, the patient received gradually increasing amounts of ACTH (caution was exercised because of the presence of glaucoma). A significant fall in eosinophils occurred but the maximal level of ketones was actually higher than during the control fast.

During fast No. 3, the patient received the usual

dosage of 100 mg. of ACTH daily (and also was pretreated with his material). He again had a progressive elevation of blood ketones which significantly exceeded that obtained during the control fast. During this period of ACTH administration, no fall in eosinophils occurred. On the other hand, there appeared to be no question that adrenal stimulation was obtained, in view of the very high 17-ketosteroid values and the evidence of marked sodium retention. It is of interest also that this patient made a minimal clinical response to ACTH administration. One might conjecture that this man's adrenals produced unusual steroids in response to ACTH treatment.

In Figure 6 is shown the response pattern in a female patient with monocytic leukemia. The characteristic suppression of hyperketonemia and hyperketonuria was observed in this patient together with an increase in urinary 17-ketosteroid excretion and urinary nitrogen excretion. No fall in circulating eosinophils was obtained, presumably because of the abnormal condition of the bone marrow in this individual.

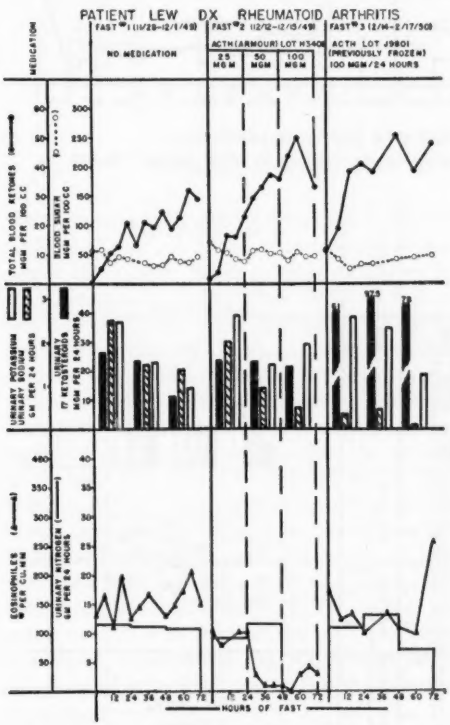

FIG. 5. FASTING STUDIES IN AN "ACTH-RESISTANT PATIENT" WITH RHEUMATOID ARTHRITIS

The findings would suggest some bizarre type of adrenal cortical response to the administered ACTH.

In Figure 7 are shown the findings in a patient with a syndrome characterized by insulin resistant diabetes, hirsutism, amenorrhea, and the lack of any spontaneous ketonuria despite a blood sugar which was constantly in excess of 300 mg./100 cc.[7] (This patient on one occasion received in excess of 20,000 units of crystalline insulin intravenously in a space of 24 hours without any fall in blood sugar occurring.)

During the control fast in this girl, the blood and urinary ketones remained at levels only slightly in excess of normal. The blood sugar values actually rose (instead of falling as is the usual observation in the control fasts). The urinary nitrogens remained low and the circulating eosinophils were at all times at a level compatible with considerable adrenocortical stimulation. Urinary 17-ketosteroids were not elevated.

During ACTH administration, it will be noted that blood and urinary ketones were actually increased over the control level. This same statement applies to the blood sugar levels and urinary nitrogen excretion. The rise in 17-ketosteroid excretion and fall in circulating eosinophils to 0 would be compatible with adrenocortical response to the administered ACTH.

This patient subsequently had an exploratory laparotomy which revealed the presence of large polycystic ovaries but failed to reveal evidence of tumor of either adrenal gland. Unfortunately the surgeon did not obtain a biopsy of the adrenal at the time of surgery. Both ovaries were removed without any detectable alteration in the clinical or biochemical status of the patient. Menopausal symptoms did not ensue, nor did the characteristics of her diabetes change.

It seems probable to us that this patient has some variety of adrenal hyperplasia secondary to pituitary pathology which accounts for all of her metabolic and clinical abnormalities.

In Figure 8 are shown the findings in a young

[7] We are indebted to Dr. Myron Arrick for the opportunity to study this patient.

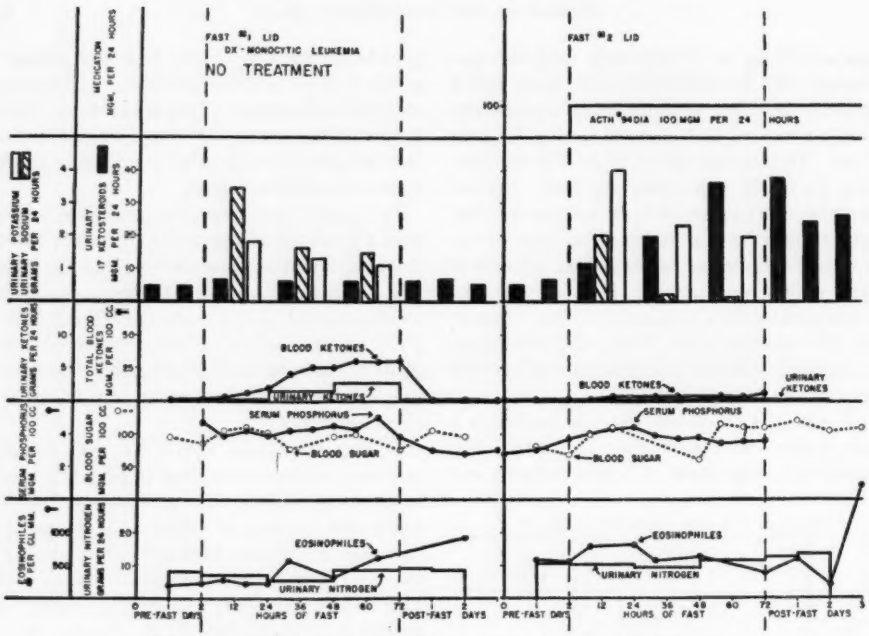
FAST #1 LID
DX - MONOCYTIC LEUKEMIA
NO TREATMENT
FAST #2 LID
ACTH #9404A 100 MGM PER 24 HOURS
MEDICATION MGM. PER 24 HOURS
URINARY POTASSIUM URINARY SODIUM GRAMS PER 24 HOURS
URINARY 17 KETOSTEROIDS MGM. PER 24 HOURS
URINARY KETONES GRAMS PER 24 HOURS
TOTAL BLOOD KETONES MGM. PER 100 CC.
BLOOD KETONES
URINARY KETONES
SERUM PHOSPHORUS MGM. PER 100 CC.
BLOOD SUGAR MGM. PER 100 CC.
SERUM PHOSPHORUS
BLOOD SUGAR
EOSINOPHILES # PER CU. MM.
URINARY NITROGEN GRAMS PER 24 HOURS
EOSINOPHILES
URINARY NITROGEN
PRE-FAST DAYS
HOURS OF FAST
POST-FAST DAYS

FIG. 6. FASTING STUDIES IN A PATIENT WITH MONOCYTIC LEUKEMIA

The effect of ACTH upon blood ketones is in accord with the observations in other patients studied.

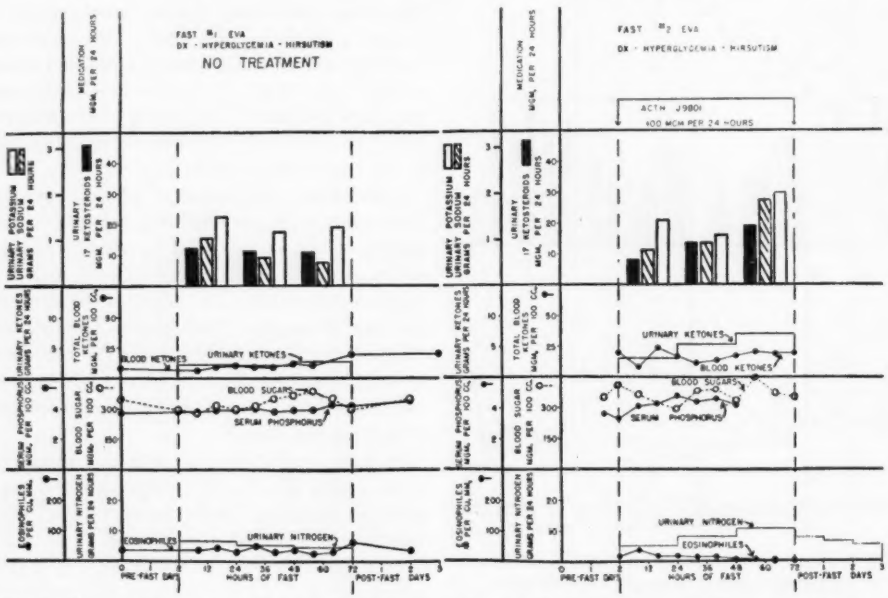
FAST #1 EVA
DX - HYPERGLYCEMIA - HIRSUTISM
NO TREATMENT
FAST #2 EVA
DX - HYPERGLYCEMIA - HIRSUTISM
ACTH J9801
100 MGM PER 24 HOURS
MEDICATION MGM. PER 24 HOURS
URINARY POTASSIUM URINARY SODIUM GRAMS PER 24 HOURS
URINARY 17 KETOSTEROIDS MGM. PER 24 HOURS
URINARY KETONES GRAMS PER 24 HOURS
TOTAL BLOOD KETONES MGM. PER 100 CC.
BLOOD KETONES
URINARY KETONES
SERUM PHOSPHORUS MGM. PER 100 CC.
BLOOD SUGAR MGM. PER 100 CC.
BLOOD SUGARS
SERUM PHOSPHORUS
EOSINOPHILES # PER CU. MM.
URINARY NITROGEN GRAMS PER 24 HOURS
EOSINOPHILES
URINARY NITROGEN
PRE-FAST DAYS
HOURS OF FAST
POST-FAST DAYS

FIG. 7. FASTING STUDIES IN A PATIENT SUFFERING FROM INSULIN-RESISTANT DIABETES, HIRSUTISM, OBESITY, AND AMENORRHEA

The findings would suggest that this patient has some variety of adrenocortical hyperplasia. She does not have Cushing's syndrome, nor does she have typical adrenogenital syndrome.

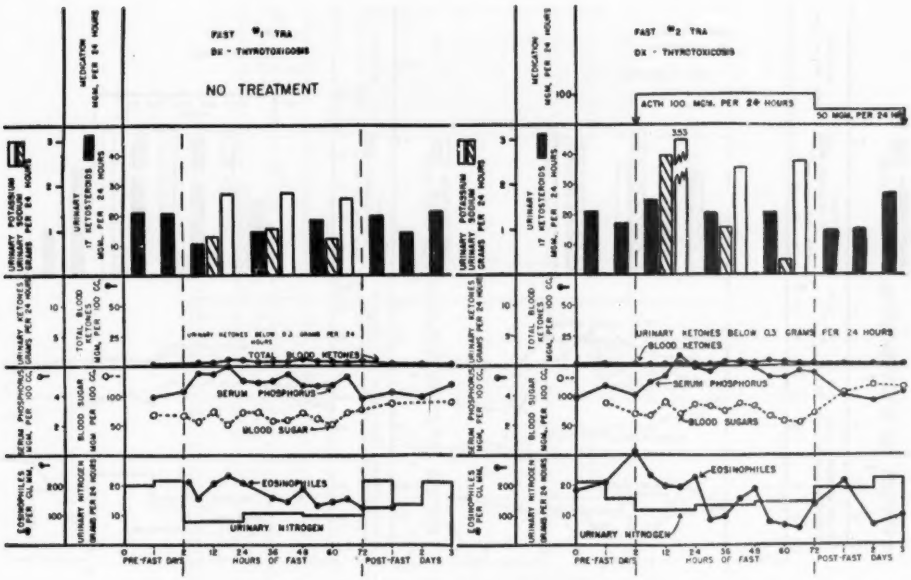

FIG. 8. FASTING STUDIES IN A YOUNG MALE PATIENT WITH THYROTOXICOSIS
Fasting with or without ACTH failed to produce a hyperketonemia in this patient.

male patient with severe thyrotoxicosis. We had anticipated that the fasting-induced hyperketonemia in this patient would be most excessive; instead, essentially no elevation in blood ketones was obtained during either the control fast or the fast during which ACTH was administered. Except for an increase in urinary nitrogen, an elevation in serum phosphorus, and a significant change in the pattern of urinary sodium and potassium and 17-ketosteroid excretion, the findings in the two fasts were essentially identical. One might postulate that a patient with thyrotoxicosis would have sufficient adrenal cortical hyperplasia to account for these findings. The relatively high eosinophil level and the rather poor response of eosinophils to administered ACTH would not agree too well with this concept. An alternative hypothesis would be that hyperthyroidism *per se* is associated with an increased rate of utilization of ketones.[8]

In Figures 9 and 10 are shown four fasting studies obtained in a female patient with active acromegaly. A number of interesting and not readily explainable changes were observed in this patient.

These findings may be summarized as follows:

1. Blood ketones. The "usual" suppression of hyperketonemia was obtained with ACTH, but apparent accentuation of hyperketonemia occurred during cortisone administration. Testosterone propionate appeared to diminish the early phase of ketone elevation, but did not modify the eventual hyperketonemia.

2. Circulating eosinophils disappeared completely in response to ACTH and fell markedly during fasting alone and during cortisone administration.

3. Serum phosphorus. As in the case of most acromegalics, this was elevated initially. It rose to extremely high levels during the control fast. It fell during the administration of ACTH, of cortisone, and of testosterone.

4. Blood sugar. This was slightly elevated initially. It fell in a marked degree during the control fast; during the "testosterone fast"; tended to

[8] Since the submission of this paper a "control" fast, carried out in a *female* hyperthyroid patient, resulted in the usual picture of marked hyperketonemia.

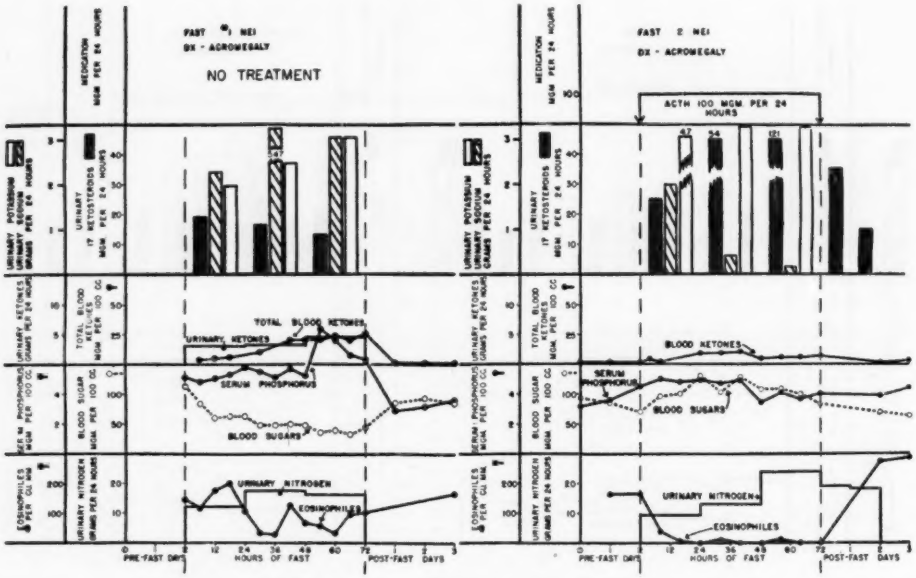

FIG. 9. FASTING STUDIES IN A FEMALE PATIENT WITH ACROMEGALY

The usual effect of ACTH upon blood ketones is observed.

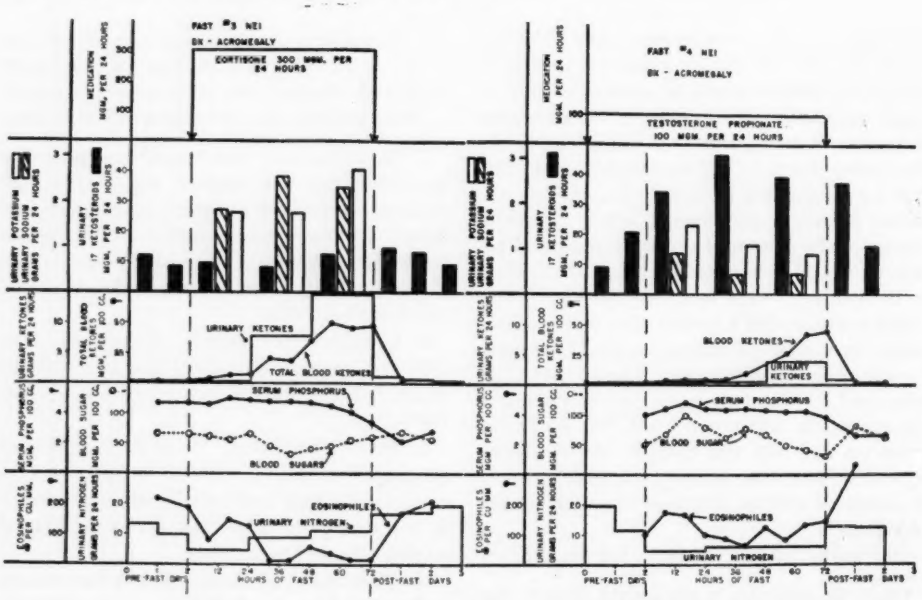
FAST #3 NEI
DX - ACROMEGALY
CORTISONE 300 MGM. PER 24 HOURS
FAST #4 NEI
DX - ACROMEGALY
TESTOSTERONE PROPIONATE 100 MGM. PER 24 HOURS
MEDICATION MGM. PER 24 HOURS
URINARY POTASSIUM URINARY SODIUM GRAMS PER 24 HOURS
URINARY 17 KETOSTEROIDS MGM. PER 24 HOURS
URINARY KETONES GRAMS PER 24 HOURS
TOTAL BLOOD KETONES MGM. PER 100 CC.
URINARY KETONES
TOTAL BLOOD KETONES
BLOOD KETONES
SERUM PHOSPHORUS MGM. PER 100 CC.
BLOOD SUGAR MGM. PER 100 CC.
SERUM PHOSPHORUS
BLOOD SUGAR
EOSINOPHILES # PER CU. MM.
URINARY NITROGEN GRAMS PER 24 HOURS
EOSINOPHILES
URINARY NITROGEN
PRE-FAST DAYS
HOURS OF FAST
POST-FAST DAYS

FIG. 10. FASTING STUDIES IN THE SAME ACROMEGALIC PATIENT

Cortisone in this patient appeared to accentuate rather than to depress the hyperketonemia. Testosterone may have delayed the elevation in blood ketones, but did not prevent the high level on the third day.

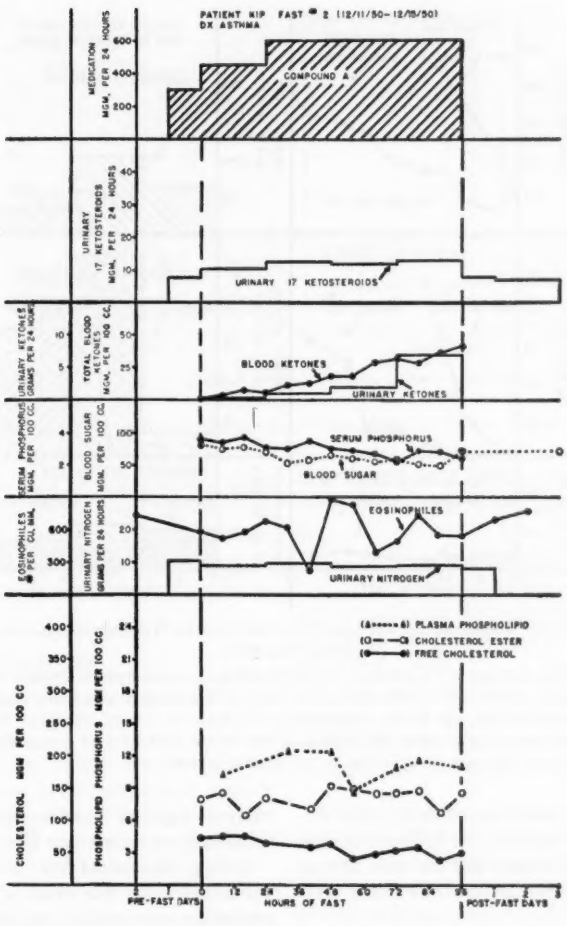

FIG. 11. EFFECT OF COMPOUND A (11-DEHYDROCORTICOSTERONE) UPON FASTING-INDUCED HYPERKETONEMIA

No metabolic effect of this preparation in a dosage of 600 mg. a day could be observed.

fall during cortisone administration; and rose significantly during ACTH administration.

5. Urinary nitrogen was highest during ACTH administration and lowest during testosterone administration, as one would expect.

6. Urinary 17-ketosteroids rose to the highest levels we have observed in anyone with this dosage of ACTH (121 mg./24 hr. during the third day of ACTH administration).

7. Urinary sodium was excreted in large amounts during the control fast and during cortisone administration; and was retained during ACTH and testosterone administration.

8. Urinary potassium was excreted in very large amounts during the administration of ACTH.

We have not been able to arrive at a satisfactory explanation for the differences in blood ketones

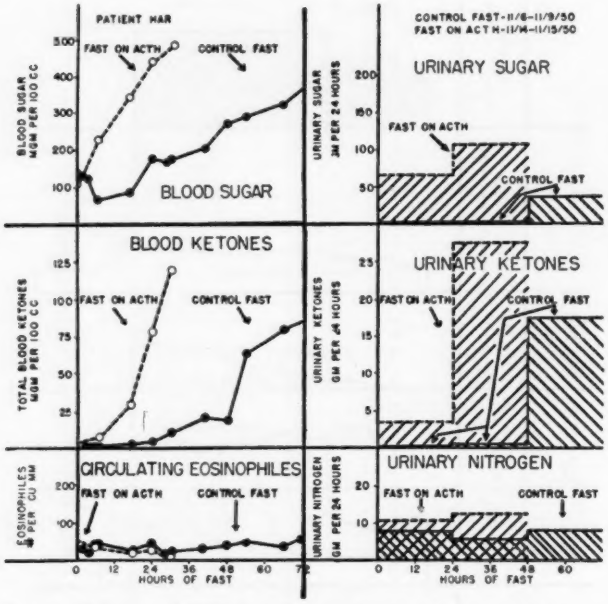

FIG. 12. EFFECT OF ACTH UPON FASTING-INDUCED HYPERKETONEMIA IN A SEVERE DIABETIC

Two findings are of interest: 1. ACTH did not prevent hyperketonemia for reasons which will be discussed elsewhere. 2. The amount of urinary sugar is greater than can be accounted for on the basis of urinary nitrogen. No emphasis is placed upon this finding because of the possibility of endogenous stores of glycogen contributing to the urinary hexose.

during ACTH and cortisone administration respectively. All other patients, so far studied who have received cortisone, have had the same ketone response to this material as has been noted with ACTH.

In Figure 11 are shown the findings during a fast associated with the administration of 600 mg. of Compound A daily. This same patient had a very usual response to ACTH administration in terms of all entities studied. One may, therefore, assume that Compound A in this dosage has no effect upon urinary 17-ketosteroids, blood ketones, serum phosphorus, blood sugar, circulating eosinophils, or urinary nitrogen.

For reasons which will be discussed presently, it was desired to determine the effect of ACTH upon fasting-induced hyperketonemia in a patient with relatively severe diabetes. In Figure 12 are shown the composite findings of an attempted study in a girl of 19 who required 80 units of insulin daily on an average diet.

During the control fast, the blood sugar which fell initially (as the result of residual effect of protamine zinc insulin) had reached levels in excess of 350 mg./100 cc. by the end of the third day. The blood ketone levels exceeded 75 mg./100 cc. at this same time.

When ACTH was administered in a dosage of 25 mg. every six hours during the second fast, an extremely rapid rise in blood sugar followed by an equally rapid rise in blood ketones occurred. The study was of necessity terminated in the early part of the second day because of the occurrence of progressive dehydration and acidosis. It is interesting that in this patient the increase in urinary sugar considerably exceeded the expected increase on the basis of urinary nitrogen (see below).

In Figure 13 are shown 20 blood ketone levels

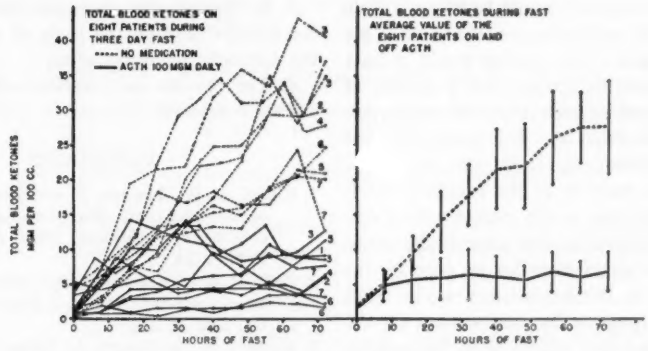
TOTAL BLOOD KETONES ON EIGHT PATIENTS DURING THREE DAY FAST
----- NO MEDICATION
—— ACTH 100 MGM DAILY
TOTAL BLOOD KETONES MGM PER 100 CC.
HOURS OF FAST
TOTAL BLOOD KETONES DURING FAST AVERAGE VALUE OF THE EIGHT PATIENTS ON AND OFF ACTH
HOURS OF FAST

FIG. 13. ACTH EFFECT UPON FASTING-INDUCED HYPERKETONEMIA (COMPOSITE OF 20 FASTS)

The suppression of fasting-induced hyperketonemia by ACTH is obvious.

obtained in eight patients during standard fasts with and without ACTH. These values were taken at random from studies on individuals without endocrinopathies. The suppressing effect of ACTH upon fasting-induced hyperketonemia is apparent.

DISCUSSION

From the foregoing data, certain deductions appear to be warranted:

1. The administration of adequate amounts of ACTH or of cortisone to the majority of fasting individuals (without pre-existing endocrinopathies) results in partial or complete suppression of the fasting-induced hyperketonemia.

2. This suppression is usually associated with an elevation of blood sugar and an increase in urinary nitrogen.

3. The total increase in urinary nitrogen is usually of small magnitude; that is, it would not appear to be adequate in amount to explain any major "fat sparing effect" during the period of fast.

4. During the administration of ACTH and cortisone, there is no decrease in energy requirement; if anything, the oxygen utilization increases.

5. From the foregoing, it would appear that the suppression of fasting-induced hyperketonemia by ACTH and certain adrenal steroids can only have a limited number of explanations. Among these would be the following:

a. That one or more adrenal steroids increase the rate of utilization of ketones by peripheral tissues.

b. That one or more adrenal steroids decrease the rate of formation of ketones from fatty acids, and at the same time increase the rate of direct utilization of fatty acids for energy purposes by peripheral tissues.

c. That certain adrenal steroids so change the metabolic pathway of fatty acids, that mid-zone metabolites other than ketones are formed. Among such possible metabolites would be hexose.

Other reports from this laboratory, dealing with the rate of utilization of intravenously administered ketones and of intravenously administered fatty acids in the human subject under controlled conditions with and without ACTH, cortisone, and other adrenal steroid administration, will subsequently be presented. It is the purpose of this paper only to indicate that such substances do, in a very significant way, influence the metabolism of fat, and to suggest some of the ways in which such metabolic modification could be accomplished.

If one examines the findings in diabetic patient Har (Figure 12), he finds that during the first two days of the "ACTH fast" the total amount of nitrogen excreted was slightly in excess of 23 gm., and that the total amount of glucose excreted was slightly in excess of 200 gm. On the basis of a D/N ratio of 3.65, the maximal amount of carbohydrate which could have been derived from

protein during this period would be 84 gm. This leaves 116 gm. of carbohydrate not accounted for by urinary nitrogen. This finding would at least be compatible with the concept that a portion of the effect of adrenal steroids is that of acceleration of neoglucogenesis from fat. It is recognized that in this patient, storage glycogen may have contributed in some measure to the excreted carbohydrate. The findings in this patient will be discussed in more detail in another paper in this series dealing with studies in diabetics on pure fat intake. Whatever the final explanation may be, there appears to be no question that one or more adrenal steroids exert profound effects upon fat metabolism.

SUMMARY

1. Total fasting over a period of three and one-half days results in significant hyperketonemia and ketonuria in the majority of subjects studied.

2. The administration of ACTH and cortisone in adequate amounts results in partial or complete suppression of such fasting-induced hyperketonemia and ketonuria.

3. It appears that such an effect can be accounted for only on the basis of modification of the metabolic pathways of fat.

4. The possible modifications which might occur are considered.

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THE EFFECTS OF ADRENALINE UPON RENAL FUNCTION AND ELECTROLYTE EXCRETION [1]

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(Submitted for publication July 26, 1951; accepted August 27, 1951)

The subcutaneous and intravenous infusion of adrenaline in man at a rate of 8 to 10 gamma per minute has previously been shown to produce a decrease in both renal plasma flow and glomerular filtration rate (1–4). The changes are not, however, proportional; and thus with a greater reduction in the plasma flow, a rise in filtration fraction results. The effects of the adrenaline-induced renal ischemia and hypofiltration on electrolyte excretion have not been adequately described. It is the purpose of this report to present data concerning the effect of intravenous adrenaline on both renal function and electrolyte excretion.

METHODS

Studies of renal function and determinations of electrolyte excretion were conducted on 12 young men without evidence of cardiovascular or renal disease. The experimental subjects received a normal hospital diet which contained between 7 and 15 grams of sodium chloride daily. The experiments were performed with the patients in the basal state after adequate hydration by the oral ingestion of one liter of water 60 to 90 minutes before the study. After three control periods of 15 minutes each, 0.45 to 0.80 mg. of adrenaline was administered by a constant injection syringe during a 45 minute period. The renal clearance studies were repeated during the administration of adrenaline. Renal plasma flow was determined by the clearance of para-aminohippurate as previously described (5). The glomerular filtration rate was measured by the clearance of inulin utilizing the method of Roe, Epstein, and Goldstein (6). The average priming solution of sodium para-aminohippurate and inulin consisted of 3 cc. and 50 cc., respectively. The average sustaining solution contained 14 cc. of sodium para-aminohippurate and 100 cc. of inulin, diluted to 200 cc. with triple distilled water. Approximately 150 cc. of sustaining solution was used for each experiment.

Sodium and potassium were determined by an internal standard Perkin-Elmer flame photometer. Chloride was determined by the titrimetric iodate method of Sendroy as modified by Van Slyke and Hiller (7).

RESULTS

The control values for renal hemodynamic and electrolyte studies are the averages of three consecutive periods. The values during the constant infusion of adrenaline are the averages of two periods beginning 15 minutes after the start of the infusion.

There was a marked and significant decrease in the mean renal plasma flow ($t = 7.82$, $P < 0.01$). In nine subjects the rate of glomerular filtration decreased, and in the other three it increased slightly. The mean change in the filtration rate was statistically significant ($t = 2.62$, $P < 0.02$). The filtration fraction increased in all patients, and the mean change was highly significant ($t = 9.32$, $P < 0.01$). The clearance data are recorded in Table I.

The serum sodium and chloride concentrations were unchanged by the administration of adrenaline. However, the serum potassium fell in each instance. The mean decrease in serum potassium was highly significant ($t = 3.90$, $P < 0.01$). Serum electrolyte concentrations are recorded in Table II.

A decrease in urinary sodium, chloride, and potassium excretion occurred in each subject. The mean change for each of the electrolytes studied was very significant ($P < 0.01$). The rate of urine flow decreased in eight subjects and increased in the other four. The mean change for the group was + 9.4 per cent. The urinary electrolyte excretion was independent of the rate of urine flow. Data for urinary electrolyte excretion are recorded in Table III.

The data presented in this report afford a means of studying the interrelationships of hypofiltration and electrolyte excretion. There was a moderate direct correlation between per cent change in uri-

[1] Published with the approval of the Chief Medical Director. The statements and conclusions published by the authors are the result of their own study and do not necessarily reflect the opinion or policy of the Veterans Administration.

TABLE I

Renal hemodynamic studies before and during adrenaline infusion

Patient	Renal plasma flow (*cc. per min.*)			Glomerular filtration (*cc. per min.*)			Filtration fraction (*per cent*)		
	Control	During adrenaline	Per cent change	Control	During adrenaline	Per cent change	Control	During adrenaline	Per cent change
W. A.	669.2	363.5	−45.7	121.4	94.1	−22.5	18.1	25.9	+43.1
K. C.	532.7	361.8	−32.1	104.0	106.3	+2.2	19.5	29.4	+50.8
L. R.	682.1	370.5	−45.7	108.7	97.1	−10.7	15.9	26.2	+64.8
R. H.	673.1	331.0	−50.8	109.8	78.0	−29.0	16.3	23.6	+44.8
D. J.	583.5	208.5	−64.3	117.3	62.5	−46.7	20.1	30.0	+49.3
A. T.	544.8	386.0	−29.2	108.0	118.8	+10.0	19.8	30.8	+55.6
G. T.	604.1	326.3	−46.0	123.8	88.7	−28.4	20.5	27.2	+32.7
O. K.	766.3	475.1	−38.0	143.7	121.7	−15.3	18.8	25.6	+36.2
R. Ho.	697.2	392.8	−43.7	122.6	100.1	−18.4	17.6	25.5	+44.9
E. R.	625.7	549.1	−12.2	122.4	128.5	+5.0	19.6	23.2	+18.4
D. B.	663.5	367.6	−44.6	110.6	101.4	−8.3	16.7	27.6	+65.3
S. L.	498.2	313.9	−37.0	92.3	72.6	−21.3	18.5	23.1	+24.9
Mean	628.4	370.5	−40.8	115.4	97.5	−15.3	18.5	26.5	±44.2
S. D.	±78.2	±83.5	±12.8	±12.9	±20.0	±16.2	±1.6	±2.6	±14.5

nary sodium and per cent change in renal plasma flow ($r = 0.65$), and a high direct correlation between per cent change in urinary sodium and per cent change in glomerular filtration (Figure 1) ($r = 0.84$). Eliminating the effect of glomerular filtration, by the method of partial correlation (8), reduced the correlation between per cent change in urinary sodium and per cent change in renal plasma flow to insignificance (partial $r = 0.46$, $P > 0.1$).

Analysis of the data on chloride excretion and renal function was similar to that for sodium but yielded somewhat lower correlations. The correlation between per cent change in urinary sodium and per cent change in urinary chloride was 0.66.

An insignificant correlation was found between per cent change in the urinary potassium and per cent change in renal plasma flow ($r = 0.55$, $P > 0.05$). Similarly, an insignificant correlation was found between per cent change in urinary potassium and per cent change in glomerular filtration rate ($r = 0.55$, $P > 0.05$). There was no correlation between the per cent change in urinary potassium and the per cent change in serum potassium

TABLE II

Serum electrolyte concentrations before and during adrenaline infusion

Patient	Serum sodium (*milli-equivalents per liter*)			Serum potassium (*milli-equivalents per liter*)			Serum chloride (*milli-equivalents per liter*)		
	Control	During adrenaline	Per cent change	Control	During adrenaline	Per cent change	Control	During adrenaline	Per cent change
W. A.	144.0	133.7	−7.2	4.02	3.91	−2.7	104.6	104.9	+0.3
K. C.	147.3	141.1	−4.2	4.72	3.62	−23.3	105.8	105.9	+0.1
L. R.	149.0	143.5	−3.7	3.83	2.64	−31.1	105.6	104.0	−1.5
R. H.	148.3	148.3	0.0	4.43	3.52	−20.5	104.4	105.9	+1.4
D. J.	146.4	146.6	+0.1	4.25	3.04	−28.5	103.9	98.1	−5.6
A. T.	142.7	143.1	+0.3	4.81	3.83	−20.4	100.5	100.0	−0.5
G. T.	143.3	143.1	−0.1	4.27	3.77	−11.7	106.6	107.8	+1.1
O. K.	149.2	149.4	−0.1	5.39	4.09	−24.1	109.5	110.5	+0.9
R. Ho.	138.3	138.2	−0.1	4.52	4.19	−7.3	104.0	104.9	+0.9
E. R.	147.5	147.7	+0.1	4.56	4.31	−5.5	101.0	102.0	+1.0
D. B.	151.8	152.0	+0.1	4.54	3.75	−17.4	104.5	104.5	0.0
S. L.	147.7	146.6	−0.8	4.53	3.83	−15.5	102.0	102.9	+0.9
Mean	146.3	144.4	−1.3	4.49	3.71	−17.3	104.4	104.3	−0.1
S. D.	±3.6	±5.1	±2.4	±0.50	±0.47	±9.1	±2.5	±3.3	±6.4

TABLE III

Urine electrolyte excretions before and during adrenaline infusion

Patient	Urine sodium excretion (*micro-equivalents per minute*)			Urine chloride excretion (*micro-equivalents per minute*)			Urine potassium excretion (*micro-equivalents per minute*)		
	Control	During adrenaline	Per cent change	Control	During adrenaline	Per cent change	Control	During adrenaline	Per cent change
W. A.	168	54	−67.9	153	55	−64.1	107	34	−68.2
K. C.	162	110	−32.1	185	114	−38.4	102	43	−57.8
L. R.	114	65	−43.0	87	63	−27.6	93	54	−41.9
R. H.	143	46	−67.8	140	28	−80.0	118	24	−79.7
D. J.	179	35	−80.5	145	30	−79.3	72	24	−66.7
A. T.	149	136	−8.7	153	105	−31.4	117	54	−53.9
G. T.	206	69	−66.5	112	45	−59.8	68	18	−73.5
O. K.	156	82	−47.4	225	94	−58.2	167	78	−53.3
R. Ho.	165	89	−46.1	178	94	−47.2	105	28	−73.3
E. R.	122	96	−21.3	108	73	−32.4	51	37	−27.5
D. B.	63	54	−14.3	49	19	−61.2	62	17	−72.6
S. L.	111	47	−57.7	139	34	−75.5	126	20	−84.1
Mean	145	74	−46.1	140	63	−54.6	99	36	−62.7
S. D.	±37	±30	±23.2	±47	±33	±18.9	±32	±18	±16.5

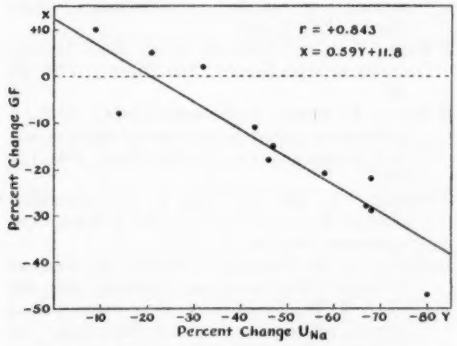

FIG. 1. CORRELATION BETWEEN PER CENT CHANGE IN GLOMERULAR FILTRATION RATE (GF) AND PER CENT CHANGE IN URINARY SODIUM EXCRETION (U_{Na})

($r = -0.01$). This pattern for potassium was not altered by applying the method of partial correlations.

DISCUSSION

There are three major factors which regulate the renal excretion of sodium and chloride; namely serum concentration, glomerular filtration rate, and tubular reabsorption. No evidence exists that these ions are cleared by tubular excretion. Under ordinary circumstances the serum concentration of these ions remains relatively constant. It is therefore apparent that this factor, *per se*, has no influence on the urinary excretion of these electrolytes. The constant infusion of adrenaline did not alter the serum concentration of either sodium or chloride, and thus this factor did not contribute to the changes in the excretion of these electrolytes.

It is impossible to assess adequately the relationship of glomerular filtration and tubular reabsorption to the ultimate excretion of sodium and chloride. Formulae which are used to express tubular reabsorption or rejection fractions are fallacious since under normal circumstances they merely express the constancy of the serum ion concentration. Furthermore, it is not possible to assess tubular reabsorption activity as an independent entity when the filtration rate is changing (9). The perplexity of the problem is manifested by the conflicting opinions expressed in the current literature (9–13).

Under the conditions of this experiment there was a high degree of correlation between hypofiltration and diminution in urinary sodium and chloride. Such a relationship could result from any or all of the following factors: (*1*) decreased load, (*2*) increased rate of tubular reabsorption, and (*3*) decreased velocity of flow in the tubular lumen. Since the serum electrolyte concentration was constant, decreased load and hypofiltration are synonymous. If hypofiltration were the sole factor and the percentage of tubular reabsorption of the filtered load were constant, then the percentage changes in glomerular filtration and urinary elec-

trolyte excretion would be comparable. However, the changes in urinary sodium and chloride were more than three fold those of filtration rate. Since hypofiltration is associated with proportionately greater changes in urinary sodium and chloride, other factors must be involved. In view of these disproportionate changes, tubular reabsorption cannot be evaluated as an independent entity (9). While increased tubular reabsorption must be at least a contributing factor, this does not necessarily infer increased specific activity of tubular cells for reabsorption of sodium and chloride. Hypofiltration may influence reabsorption by increasing the time of contact of electrolytes with tubular cells.

The problem of evaluating potassium excretion is much more complex than that for sodium and chloride since both tubular reabsorption and tubular excretion are involved. With the infusion of adrenaline the problem is further complicated by a decrease in the serum concentration of potassium. The alteration in serum potassium is not, however, correlated with the changes in renal function nor with urinary excretion, and must reflect an intracellular shift. A similar decrease in serum potassium has been noted by Keys following the injection of epinephrine (14). This author also noted a return above pre-injection levels within 50 minutes. It is to be noted that our studies were carried out during the constant infusion of epinephrine, and the serum potassium was not determined subsequently. The decreased urinary excretion of potassium during adrenaline infusion cannot be adequately explained by either the changes in serum concentration or by hypofiltration.

CONCLUSIONS

1. The constant intravenous infusion of adrenaline in doses of 10 to 18 gamma per minute caused a decreased renal plasma flow, a decreased glomerular filtration rate, and an increased filtration fraction.

2. There was a decrease in the urinary excretion of sodium, chloride and potassium. The serum sodium and chloride were not altered but the serum potassium decreased.

3. A high degree of correlation was demonstrated between the percentage changes in urinary sodium and chloride and the percentage change in glomerular filtration rate. This was not true for potassium.

4. Tubular reabsorption could not be evaluated in the presence of reduced filtration rate. The reasons for this have been discussed.

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A QUANTITATIVE RELATIONSHIP BETWEEN THE WATER CONTENT OF SKIN AND INFLAMMATION PRODUCED BY CHEMICAL IRRITANTS [1]

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Frequently it is desirable to determine quantitatively the inflammatory response of tissues to chemical irritants. Until now the approach to this problem has depended chiefly upon subjective analysis of the degree of redness or swelling of inflamed tissues, such as that reported by Munch (1). Technics which depend to a considerable extent upon the investigator's visual evaluation possess certain recognized inherent errors. Even the unintentional bias of the investigator may significantly influence the assignment of a specific value to a visually observed physiological phenomenon.

Oettel (2) has described a method of accurately determining the redness associated with inflamed tissue. However, a quantitative relationship between redness and inflammation has not been firmly established. More recently Weatherby (3) has utilized the phenomenon of trypan blue fixation at sites of inflammation. The color depth of the trypan blue deposited in inflamed sites is visually compared with color standards, and a quantitative relationship between trypan blue fixation and inflammation has been found to exist over a limited range. This technic appears to be extremely sensitive to hydrogen ion concentration and is not entirely satisfactory for irritants which produce tissue destruction.

To obviate completely subjective analysis, a new basis for determining the degree of inflammation was sought. It is generally agreed that a fundamental change in inflammatory processes is manifested by increased capillary permeability and passage of fluid into the injured area. The following study was conducted to determine whether the fluid content of an area is proportional to the intensity of inflammation. If this relationship exists, a measurement of the water content by totally objective procedures should afford a potential method of determining quantitatively the response of tissue to chemical irritants.

[1] This study was aided by a grant from the Ethicon Suture Laboratories, Inc.

[2] Ethicon Fellow, University of Cincinnati.

EXPERIMENTAL METHOD

Hair from the ventral surface of five-pound white male rabbits was removed with electrical clippers (Oster model A-2 animal clipper with No. 40 cutting head). The skin was thoroughly cleansed with a dilute solution of tincture of green soap and rinsed with distilled water. Selected sites were injected intradermally using syringes of 0.25 ml. capacity and hypodermic needles of 25 to 27 gauge. Injections were of 0.05 ml. amounts. Rabbits were sacrificed 24 hours after injection. An area one inch square surrounding the injected site was marked in ink and lightly scored with a scalpel to facilitate accurate excision. After all areas had been demarcated, the dermis layer was cleanly separated from the underlying subcutaneous tissue and transferred to weighing bottles which had been previously weighed. The weight of the wet tissue was determined. The samples were then heated under 28 to 29 inches of vacuum in an oven at 70° C. for 18 hours. The samples were cooled and reweighed to obtain the weight of dry tissue and to determine the quantity of water present in the initial specimen. A factor to be designated as W was computed.

$$W = \frac{\text{Weight of water (g.)}}{\text{Weight of dry tissue (g.)}} \times 100.$$

The effect of three irritants, 360E, Sovasol 35, and hydrochloric acid, was studied by this procedure. Both 360E and Sovasol 35 are non-polar materials which have been employed in the sterilization of catgut sutures and were found by the usual technic to possess potent inflammatory properties. Both fractions have a specific gravity of 0.75 and are water insoluble; however, up to 2 per cent emulsions may be uniformly dispersed in isotonic saline which contains two drops of Aquet/100 ml. (Aquet is a commercial non-ionic surface active agent.)

A stock solution containing two drops of Aquet/100 ml. of isotonic saline was prepared. Concentrations of 0.5, 0.75, 1.0, 1.5, and 2.0 per cent of 360E and Sovasol 35 were prepared by volume in the stock solution.

Six rabbits were used to test each irritant. Six sites were injected in each rabbit, one with the control solu-

TABLE I

W and per cent change in W

Rabbits 9930 and 9931

Injected with different concentrations of 360E

Rabbit	Control	Concentration 360E (by volume)									
		0.5 per cent		0.75 per cent		1.0 per cent		1.5 per cent		2.0 per cent	
		W	Δ*W*(%)	*W*	Δ*W*(%)	*W*	Δ*W*(%)	*W*	Δ*W*(%)	*W*	Δ*W*(%)
9930	233	333	42.8	328	40.7	424	82.1	429	84.2	456	95.6
9931	123	177	43.9	212	72.3	237	92.7	233	89.4	302	145.5

tion and the other five with each of the concentrations. The sites for injection of emulsions at each concentration were varied from one animal to another. The control injection of the stock solution (isotonic saline containing Aquet) was also made at sites similarly varied.

To determine the influence of tissue destruction and the osmotic effect produced by a chemical agent on the tissue water content, the actions of hydrochloric acid and of equimolar concentrations of sodium chloride were studied. From constant-boiling hydrochloric acid, solutions of 0.1, 0.5, 1.0, 2.5, and 5.0 per cent concentrations were prepared in distilled water. Equimolar concentrations of sodium chloride in distilled water were similarly prepared. Six sites were injected in each of six rabbits with the three lowest concentrations of hydrochloric acid solutions and the three osmotically equivalent solutions of sodium chloride. Equimolar solutions were injected in bilaterally symmetrical positions. In the same manner each of six rabbits was injected with 2.5 and 5.0 per cent hydrochloric acid solutions and the corresponding sodium chloride solutions.

RESULTS

All sites injected with Sovasol 35 and 360E appeared red and swollen, and a general trend of increased reaction with increased concentrations of irritant could be discerned. Control areas showed no evidence of inflammation. With one exception, *W* values of the control areas ranged between 220 and 296. The *W* values for areas injected with irritant were invariably greater than those of the corresponding control.

The control values of *W* and the *W* values ob-

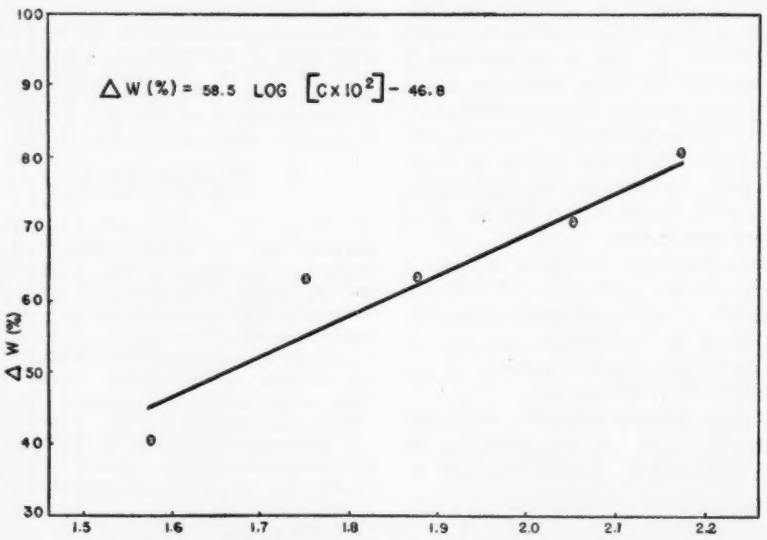

FIG. 1. LOG [CONCENTRATION SOVASOL 35 (GRAMS) × 10^2]

TABLE II

Linear coefficients and statistical data

Irritant	a (intercept)	b (slope)	Linear regression	Residual regression	Total variability	F value	Standard deviation from regression (*per cent*)
Sovasol 35	−46.8	58.5	778	85	863	27.5	4.7
360E	−103.4	90.1	1,846	79	1,924	71.9	5.4
Hydrochloric acid	−40.3	42.4	3,102	68	3,170	135.5	4.9

tained at a particular concentration were subjected to regression analysis. Results indicated that a statistically significant relationship existed between the state of hydration of the essentially normal control sites and the response of the tissue to the chemical irritant. This relationship may be demonstrated by the two animals in the series in which the W values were widely divergent (Table I). Expression of the results in per cent change in W with respect to the control value indicates that the relative changes in W with change in concentration are comparable when the influence of normal biological variations is considered. For this reason the criterion adopted in this study was the per cent change in W rather than the absolute value of W.

The skin of animals injected with hydrochloric acid showed definite necrotic changes at sites injected with 5 per cent solutions. External evidence of inflammation was observed at sites injected with the three highest concentrations of hydrochloric acid. Areas injected with 0.1 and 0.5 per cent concentrations of hydrochloric acid did not exhibit any visually apparent signs of inflammation. The W values of sites injected with hydrochloric acid were, however, appreciably greater than the values obtained for the corresponding control areas. The concentrations of sodium chloride employed did not produce significant changes in W.

The per cent change in W with respect to the control site was computed. The mean of the change at each concentration was plotted against the logarithm of the concentration of irritant

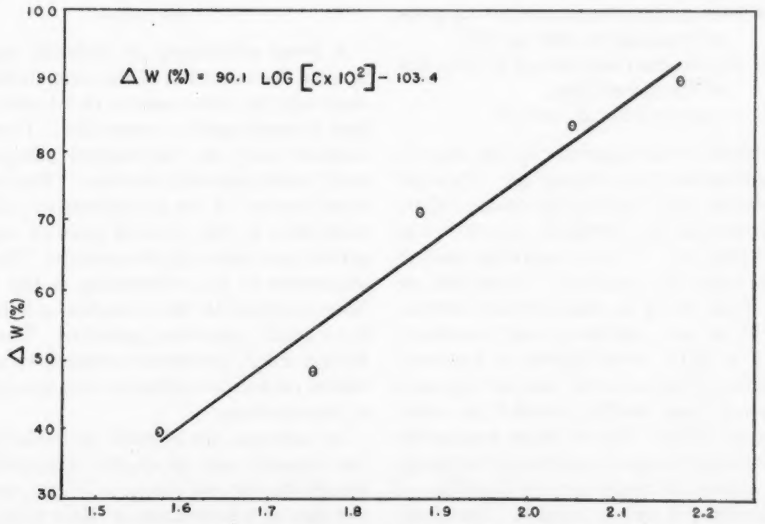

FIG. 2. LOG [CONCENTRATION 360E (GRAMS) × 10^2]

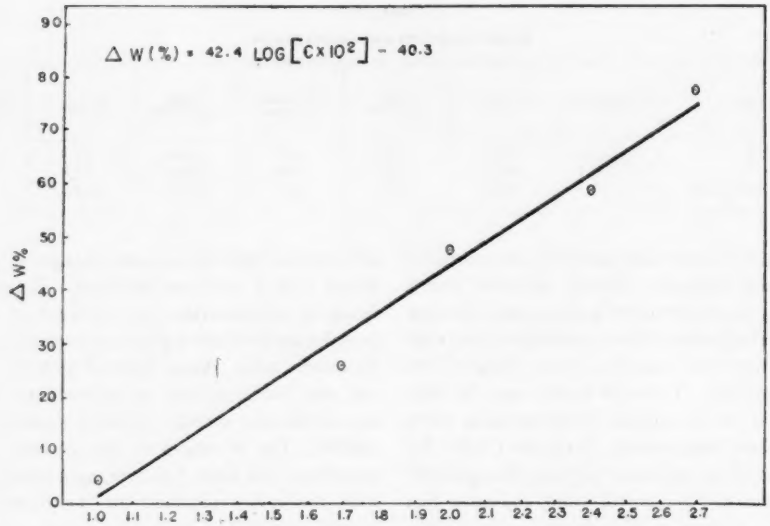

FIG. 3. LOG [CONCENTRATION HCL (GRAMS) × 10^2]

(grams) and a straight line relationship of the form:

$$\Delta W(\%) = a + b \text{ (log C)}$$

was obtained, where

$\Delta W(\%)$ = per cent change in W,
a = a constant determined by the point of intercept on $\Delta W(\%)$ axis,
b = a constant determined by the slope of the plotted line,
C = concentration of irritant.

By the method of least squares (4), the slope of the line and intercept was determined. To avoid the computation with negative logarithmic values, each concentration was multiplied by 10^2. The data were subjected to linear regression analysis and the F value was computed. These data are shown in Table II for the three irritants studied.

At the 95 per cent confidence level, the tabular value of F is 10.13 (three degrees of freedom). The computed F value for the lines of regression of the three irritants studied exceeded this value. These results indicate that a linear relationship of statistical significance exists between the change in water content of tissue and the logarithm of the concentration of irritant injected. The standard deviation from regression was approximately 5 per cent. The mean of six values at each concentration and the straight line derived by formulae from these data are shown in Figures 1, 2, and 3 for Sovasol 35, 360E, and hydrochloric acid, respectively.

DISCUSSION

A linear relationship of statistical significance between the logarithm of the concentration of irritant and the water content of injected sites has been demonstrated experimentally. These results correlate with the fundamental changes which occur in inflammatory reactions. The increase in water content of the experimentally induced inflammation by the chemical irritants may be regarded as an index of inflammation. The primary importance of this relationship is that it establishes a method for the evaluation of inflammation by a totally objective procedure. Further, the method should provide for comparisons of inflammation produced by different irritants over a range of concentrations.

As indicated, the response of tissue to an irritant depends upon its degree of hydration. Although the per cent change in W was comparable, the sites of inflammation in rabbit 9930 appeared markedly more inflamed than the corresponding

sites of rabbit 9931 (Table I). Attention is called to the fact that hydrochloric acid in low concentrations produces significant physiological changes, manifested by the increase in water content over that of controls, which are nevertheless unattended by visually apparent changes. For this reason the reliability of measuring inflammation by gross visual examination is questionable.

The study described was specifically designed to investigate the relation between chemically induced inflammation and the water content of tissue. Further investigation is indicated to determine whether a variety of compounds obey the formula $\Delta W\ (\%) = a + b\ (\log C)$. It would be of importance to determine also whether the concentration value obtained by extrapolation of the straight line to the abscissa (zero change in W) represents the concentration of irritant on the borderline of producing inflammation. If this be the case, an objectively determined numerical concentration value may be assigned to a chemical irritant and recorded in the literature as the minimal inflammatory concentration.

SUMMARY

The relation between water content of dermal tissue and inflammation produced by chemical irritants was investigated. A linear relationship of statistical significance was found to exist between the per cent change in water content and the logarithm of the concentration of irritant injected for three irritants studied. The change in water content in response to an irritant is regarded as an index of inflammation. These findings offer a potential method of quantitatively determining inflammation by a completely objective procedure.

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FERROKINETICS IN NORMAL PERSONS AND IN PATIENTS HAVING VARIOUS ERYTHROPOIETIC DISORDERS [1]

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It is possible to detect by external means the movement of Iron-59 within the human body. Individuals with certain hematopoietic disorders are thought to have abnormalities in the turnover mechanism of labelled iron. Plasma and red cell turnover of radio iron have been the topic of a previous report (1). A group of patients with various hematologic disorders have been studied by an Fe^{59} *in vivo* technique, and although each disease is represented by too few cases to make generalizations, the deviations from normal of iron turnover of liver, spleen, and bone marrow are so remarkable that a report of the methods and findings was thought desirable.

These studies have been made possible by the recent construction of reliable high-efficiency scintillation counters. Detailed descriptions of the construction and performance of these counting devices have been published by Anger (2, 3).

METHODS AND MATERIALS

Counting equipment

(*a*) *In vivo counter.* Figure 1 illustrates the counting head used for *in vivo* counting. It employs a thallium-activated sodium iodide crystal, a 2.5 cm. cube, which lies in contact with the end window of a photomultiplier tube (RCA 5819). The side of the crystal toward the patient was shielded against beta radiation.

A hydraulic lift was used to provide vertical mobility in a horizontal plane within a limited range. An adjustable height stool for the patient added to the range of positioning. The counter output was coupled to the scaler unit, which was of the usual design. The unit also contained the regulated high voltage supply for the photomultiplier tube.

The integrating circuit in connection with a Leeds and Northrup Speedomax recording millivoltmeter comprised a point-plotting linear counting rate recorder with several ranges. The numerous body positions counted and the length of time of the studies required considerable cooperation by the patient and close surveillance by the operator. A mechanized multiple port *in vivo* counter with simultaneous multichannel recording is now being constructed so that bed patients can be studied and ambulatory patient investigations facilitated.

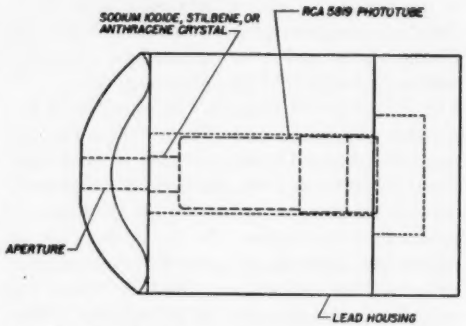

FIG. 1. THE SCINTILLATION COUNTER HEAD USED IN *IN VIVO* COUNTING

(*b*) *The vial counter.* Figure 2 shows the crystal counter arrangement for receiving the 4 ml. screw cap vials (Kimble glass 60910) containing cell or plasma

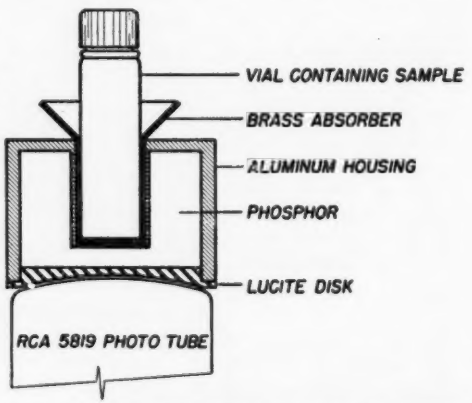

FIG. 2. CRYSTAL-PHOTO TUBE DEVICE FOR BLOOD SAMPLE ANALYSIS

[1] This work was supported in part by the Atomic Energy Commission.

samples. This assembly provided a highly efficient geometry. The operation of the phototube with less than 1 count per minute of the background attributable to phototube "noise" was satisfactory for this work. The tube was operated at less than maximum voltage and cooling or special circuits were not necessary. The shielding consisted of 6 mm. of brass housing, 5 cm. of iron, and 10 cm. of lead.

The overall efficiency of the vial counter is 17.5% for Iron-59 gammas. This is approximately the efficiency attained with the usual end window G-M tubes for betas of Fe^{59} when electrodeposited samples are analyzed. The saving in time and effort of this method over the old method of analyzing Fe^{59} is extraordinary.

The iron isotope was obtained by deuteron bombardment of cobalt in the 60-inch cyclotron in Berkeley and by a neutron bombardment of enriched Fe^{58} from Oak Ridge (4). The cyclotron-produced iron is free of Fe^{58} and only this was used in normal subjects. The most recent cyclotron-bombarded samples have had high specific activity, 10 microcuries per microgram Fe, because of the careful chemistry in preparation of the target and the separation chemistry following the exposure.[2] The samples from the enriched Fe^{58} have a slightly lower specific activity and contain small amounts of Fe^{59}.

The tracer was kept as a ferric chloride solution. An aliquot of this solution was prepared by reduction with a small amount of thioglycolic acid and neutralization with dilute sodium hydroxide to a pH of approximately 7. This mixture was added to an aqueous solution of the Globulin IV-7, Cohn [3] and was allowed to stand overnight. The iron binding capacity of this protein was measured by a method similar to that used by Surgenor, Koechlin and Strong (5). Usually only about one-half of the available unsaturation was utilized. The mixture was then filtered through a Seitz filter and transferred to sterile serum vials.

The amount of Fe^{59} injected varied from 7 to 45 microcuries. Because of radio decay, the total amount of iron and protein given each subject varied considerably; however, in no instance was an amount given which would detectably change the total plasma iron concentration. Thus true tracer amounts of iron were always used.

Injection and blood sampling

The tracer-globulin solution was injected into the antecubital vein as rapidly as possible. Blood samples were taken at approximately one-fifth, one-half, three-fourths, one and one-half, three, five and 12 hours during the first day. During the ensuing two weeks, from seven to ten samples were taken. The blood was centrifuged at 2,500 r.p.m. for 30 minutes and the packed cell volume recorded. From most of the normal subjects, as well as many of the patients, all specimens from the first four hours were analyzed for total plasma iron by the method of Kitzes, Elvehjem and Schuette (6). No changes in the total iron were noted which would significantly alter the calculated turnover (1). From all blood samples 2 ml. of plasma were pipetted to the 4 ml. medicine vials for radio analysis in the apparatus shown in Figure 2. The cells from early samples were washed in saline two or three times. After the last washing, an amount of saline equal to the packed cell volume was added to the centrifuge cone. Four ml. of the cell suspension were pipetted to the medicine vials which were then centrifuged, and the 2 ml. of cells analyzed in the same manner as the plasma samples.

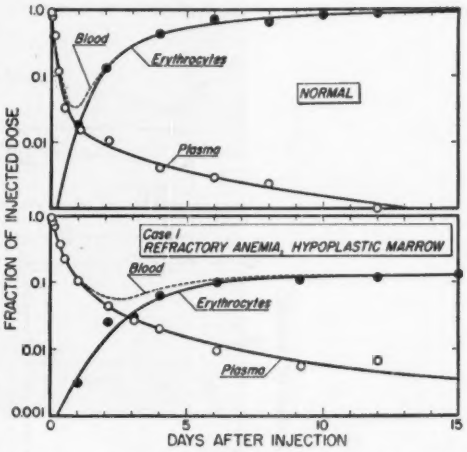

FIG. 3. FRACTION OF Fe^{59} DOSE IN PLASMA ERYTHROCYTES AND BLOOD AS A FUNCTION OF TIME AFTER A SINGLE INTRAVENOUS INJECTION

Plasma radioactivity data were plotted on semilogarithmic paper, and the extrapolated concentration at zero time was used to determine the plasma volume. Total blood volume and red cell volume were computed using this plasma volume and the average hematocrit from the first day's samples. The fraction of Fe^{59} in red cells, plasma, and blood, calculated from the volumes and concentrations of Fe^{59}, were plotted as a function of time as illustrated in Figure 3.

Body surface counting rates

Four principal positions were used in counting tissue volumes thought to be representative of blood, spleen, marrow, and liver. With the subject sitting, the counting aperture was placed against the skin. The precordium was used as the site most likely to reflect changes in a representative volume of blood. A left oblique placement over the costal cartilages with the counter pointed in the direction of the mid-mediastinum was chosen in

[2] We wish to thank Mr. Rayburn Dunn of the Radiation Laboratory, University of California, for the radiochemistry.

[3] The Globulin IV-7 was provided through the generosity of Cutter Laboratories, Berkeley, Cal.

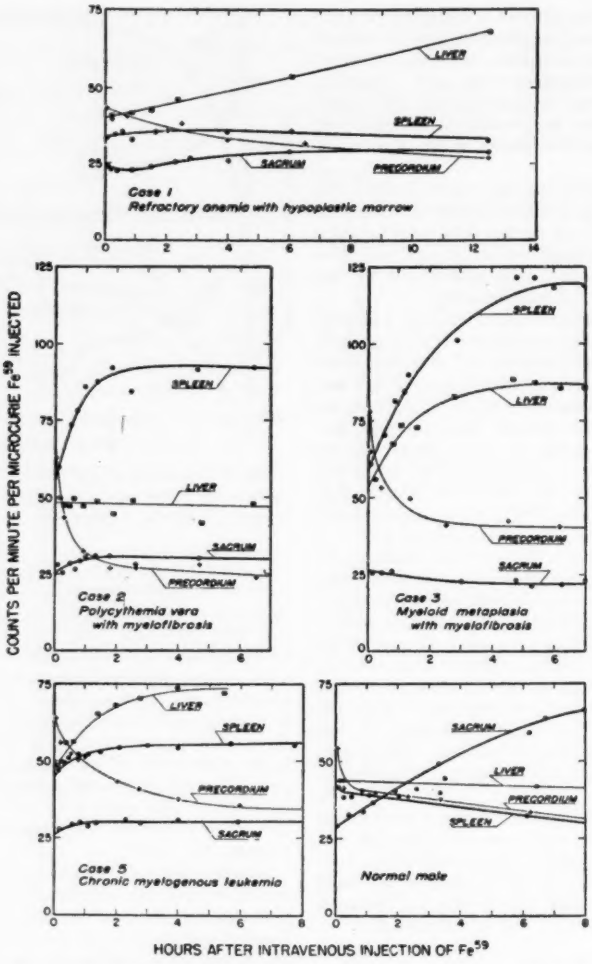

FIG. 4. GROSS BODY SURFACE COUNTING RATES AS A FUNCTION OF TIME AFTER A SINGLE INTRAVENOUS INJECTION OF Fe^{59}

order to minimize the contribution of radiation from marrow either in ribs, vertebrae, or sternum.

The right posterior axillary line between the ninth and tenth ribs with minor variations as percussion indicated, was the position used as representative of liver. In this instance, the counter was pointed anteriorly and medially so that the counting axis passed through the anterior midline at the skin surface. An analogous position for a spleen tissue volume was chosen on the left side except when the spleen was palpable. Palpable spleens were counted through the anterior or lateral abdominal wall.

For a site overlying a representative volume of marrow, the counter was placed over the sacrum and pointed directly anterior, with the patient standing. The extensive and careful investigations of Mechanik (7) as well as the contributions of others concerning the distribution of red bone marrow in the body indicate that the vertebrae and the pelvis contain the major portion in the adult. The sacrum was estimated by Mechanik to contain approximately 100 gms. of hematopoietic marrow, and this, of course, is found in a small volume relatively near the body surface. It was for this reason, as well as the fact that the marrow volume within the cone of acceptance of the counter crystal would be relatively free of spleen

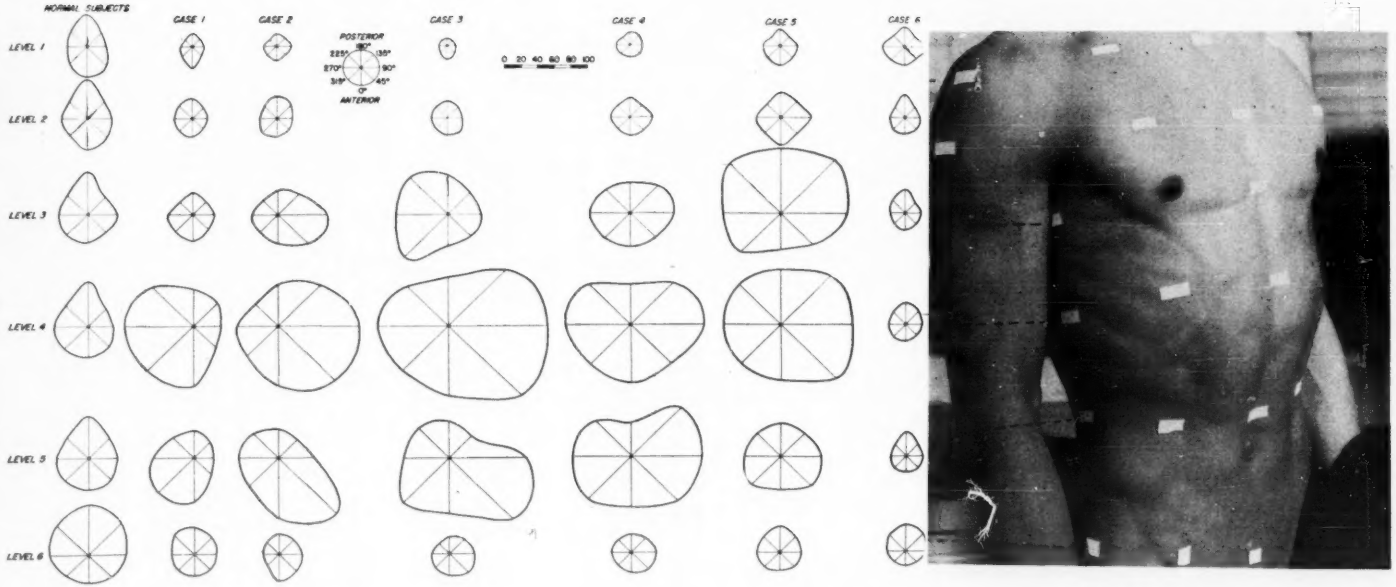

FIG. 5. GROSS BODY SURFACE COUNTING RATES (TORSO SURVEY) AT A TIME WHEN THE BLOOD RADIOIRON CONCENTRATION WAS MINIMAL

and liver influence, that the sacral position was chosen to determine marrow counting rates as a function of time.

The level used for the sacrum was located by palpation of the posterior spines of the ilium. Counting rates varied considerably with the areas and with time. Usually the range was from 500 to 5,000 counts per minute. Each position was counted long enough so that at least 1,500 counts were recorded—usually more counts than this were recorded.

A typical study was as follows: The various positions to be counted were marked out on the patient's body, and body background counting rates were determined. The injection was made with the patient sitting in the precordial position and an initial count made over the precordium; then the other positions were counted subsequently as rapidly as possible. If the changes were very slow, for example, a plasma Fe^{59} half-time of five hours, readings were made at approximately hourly intervals; however, if the half-time was short, for example, 10 minutes, positions were counted as rapidly as was possible. As indicated above, these changes with time of the body surface counting rates were recorded on a Leeds and Northrup Speedomax recorder. For more careful analysis these records were usually plotted again on semilogarithmic paper as a rate per microcurie injected. The curves of the marrow, liver, and spleen site counting rates were extrapolated to zero time, and this extrapolated point was taken as the "blood background body surface counting rate" and used in correcting the "gross body surface counting rate" to a count which represented the "tissue body surface counting rate." It was assumed that the entire counting rate at extrapolated zero time represented radioactivity in the blood of the underlying tissue and that the contribution of blood in the tissue to the counting rate was proportional to the amount of the radioiron in the blood at the time (Figure 3). Thus:

$$G_t - [G_{t=0}\ (B_t)] = T,$$

where G_t is the gross body surface counting rate at any time; $G_{t=0}$ is the gross body surface counting rate at extrapolated time zero; B_t is the fraction of Fe^{59} dose in blood at any time; and T is the tissue body surface counting rate.

Torso survey at the time of minimal blood tracer concentration

When the changes in the body surface counting rates over the three tissue sites were minimal and the blood tracer concentration had declined to a minimum (Figure 3), skin surface counting rates were obtained from many points on the torso and, in some instances, from the limbs. In order that there would be uniformity in the selection of points for counting, the following procedure was used:

With a wax pencil the following six body levels were marked on the subject:

1. The suprasternal notch
2. 2 cm. below the manubriosternal juncture
3. Fourth costosternal junction
4. 2 cm. below the xiphisternum
5. 2 cm. above the umbilicus (unless the abdomen was pendulous; then midway between levels 4 and 6)
6. 2.5 cm. below the anterior superior spine of the ilium.

Eight counting points were used at each of these levels. In each instance the counter receiver was pointed at a curved axis just anterior to the vertebral column. The anterior position was in each instance considered 0° and the posterior 180°, the left and right midaxillary lines being 90° and 270°, respectively. All *in vivo* counting rates were plotted as counts per minute per microcurie injected. The data from the multiple torso examination were plotted on polar coordinates as illustrated in Figure 5. From two to three hours were required for taking the counting rates at these 48 points. During this time there was usually very little change in the combined cell and plasma levels, which often represented less than 0.05 of the administered dose (Figure 3).

Problems associated with in vivo counting

Among the major problems which have existed in the past is the necessity for large doses of tracer because of the low efficiency of G-M tubes for gamma rays. Usually these doses have exceeded the maximum allowable tissue dose of radiation, at least instantaneously. Now, however, with the use of equipment such as described here, with an efficiency 55 times greater than similar G-M tube equipment, it is possible to restrict the tissue radiation to less than the maximum allowable dose rate. Moreover, the rise in background counting rate is not nearly proportional to the rise in efficiency: in fact, there is an increase in the ratio $\frac{\text{efficiency}}{\text{background counting rate}}$ of 11 compared to similar G-M equipment.[4]

Ideally the data accrued from an *in vivo* Fe^{59} study should be applicable to the determination of rates of movement of iron from one body compartment or tissue to another. Tobias (8) has pointed out a method whereby rate constants can be calculated from a given set of such data; however, it requires that all of the tracer be accounted for at several times. Any other method depends on estimation and approximation. The difficulty of accounting for all of the tracer at several times by *in vivo* methods is apparent when some of the variables are considered. In a particular subject, with the counter at a carefully selected site, so as to discriminate against compartments having a different metabolism, many of the variables become constants. For example, with good reproduction of positioning in the same patient, radiation absorption of the intervening tissue becomes constant. Another major variable is the fraction of the tissue or compartment being studied which the counted volume represents. This

[4] The efficiency mentioned above for the *in vivo* counter is 2.9 times that of the crystal used at the beginning of the present studies. For convenience in comparisons between patients, all graphs included in this paper are plotted in counts/minute/microcurie at the old efficiency.

also can be controlled in a single subject study so that it is a constant. Even though the turnover of tracer iron results in rather discrete separation into compartments, the problem of distinguishing these individual compartments may be great. For example, it is impossible to shield completely a gamma-sensitive device so that the counting rate of the conical tissue volume is not influenced by radiation from other areas. This variable may be eliminated by a "counter" background value taken at each time with the "counter" aperture shielded in the same manner as the "crystal."

Despite these hindrances to approaching the ideal situation in the analysis of iron *in vivo* tracer data, the uniformity of the data from normal subjects and their comparison to the abnormal affords a description of diagnostic patterns which can be useful in the classification and understanding of erythropoietic function and iron kinetics in normal and pathologic states.

Cases studied

Normal subjects: five normal male adults, ages 26 to 32; one normal female, age 31; and one normal male, age 72; were studied.

Abnormal cases: These were of varying age and sex and had refractory anemia, hypoplastic marrow (Case 1); polycythemia vera with myelofibrosis (Case 2); myeloid metaplasia with myelofibrosis (Case 3); chronic myelogenous leukemia and polycythemia vera (Case 4); chronic myelogenous leukemia (Case 5); multiple myeloma with hemolytic anemia (Case 6); refractory anemia with a hyperplastic marrow (Case 7); secondary polycythemia, polycythemia vera, and chronic lymphatic leukemia.

Synopses of case reports of patients whose Fe^{59} data are described in most detail are presented.

Case 1, J. M., refractory anemia, hypoplastic marrow

A 57-year-old married white man, J. M., was referred with the diagnosis of aplastic anemia. History was obtained of an anemia refractory to all the usual methods of therapy and requiring four to five 500 ml. transfusions per month since detection in April, 1947.

Physical examination: Physical examinations failed to disclose any notable findings except moderate bronzing of the skin.

Laboratory examinations:

RBC	2.96–4.02 million per cu. mm.
Hematocrit	27–35
Hemoglobin	8.6–10.9 gms. per 100 ml.
Platelets	140,000–218,000 per cu. mm.
Reticulocytes	0.1–0.9%
WBC	8,000–13,600 per cu. mm.
Segmented neutrophilic granulocytes	51–81%
Lymphocytes	20–49%
Monocytes	2–13%
Eosinophilic granulocytes	1–3%
Fecal urobilinogen excretion	75 mg. per day.

Sternal marrow aspiration on February 20, 1950, showed only 5.8% of the nucleated cells to be of the erythroid series.

Fe^{59} studies were started June 15, 1950.

Course: The course of the disease has been constant. Repeated transfusions have been necessary. Recovery following appendectomy was prolonged and febrile.

Case 2, E. P., polycythemia vera with myelofibrosis

A 41-year-old married woman, E. P., was referred with the diagnosis of polycythemia vera. She had had no radiation therapy.

Physical examination: Physical examination disclosed facial plethora, conjunctival injection, and a spleen so large it almost entered the pelvis.

Laboratory examinations:

RBC	6.72–8.66 million per cu. mm.
Hematocrit	61–68
Hemoglobin	14.2–17.7 gms. per 100 ml.
Platelets	110,000–300,000 per cu. mm.
Reticulocytes	1–4%
WBC	20,000–38,000 per cu. mm.
Segmented neutrophilic granulocytes	48–63%
Band neutrophilic granulocytes	8–19%
Metamyelocytes	0–6%
Myelocytes	4–15%
Progranulocytes	0–2%
Myeloblasts	0–2%
Eosinophilic granulocytes	3–8%
Basophilic granulocytes	1–13%
Lymphocytes	4–15%
Monocytes	0–2%
Nucleated red cells	0–5 per 100 WBC
Uric acid	5.0 mg. %
Total protein	8.0 gms. per 100 ml.
Albumin	4.4 gms. per 100 ml.
Globulin	3.6 gms. per 100 ml.
Fecal urobilinogen excretion	158 mg. per 24 hours.
Blood volume, with P^{32} labeled cells, was 5851 ml. (97.5 ml./kg.)	
Total red cell volume	3276 ml. (54.6 ml./kg.)
Plasma volume	2457 ml. (40.9 ml./kg.)

Aspiration specimens of the bone marrow were unsatisfactory.

A report on the sternal marrow biopsy was as follows: "The marrow shows a marked, diffuse fibrosis. Numerous megakaryocytes are seen. There is a fair number of lymphocytes, eosinophils, fairly well-matured cells of the myeloid series. Diagnosis: Myelofibrosis." No treatment was given and the course is not changed. Fe^{59} studies were started June 28, 1950.

Case 3, H. E., myelofibrosis with myeloid metaplasia

A 47-year-old married woman, H. E., was referred with the diagnosis of myelocytic leukemia. She had been given deep roentgen therapy to an enlarged spleen (87 r) on July 28, 1950. On August 4 this dose had been repeated, and on August 11, 1950, 44.4 r were given. The spleen size was reported to be smaller than on August 14, just before coming to this clinic. At no time was any radiation directed to the marrow.

Physical examination: The spleen was enlarged and the lower border was at the umbilicus.

Laboratory examinations:

On August 23, 1950, the blood count was:

RBC	4.68 million per cu. mm.
Hemoglobin	10 gms. per 100 ml.
Platelets	130,000 per cu. mm.
WBC	13,800 per cu. mm.
Reticulocytes	3.5%
Segmented neutrophilic granulocytes	47%
Band neutrophilic granulocytes	16%
Myelocytes	8%
Progranulocytes	3%
Myeloblasts	2%
Lymphocytes	23%

Sternal marrow aspirations on two occasions contained only peripheral blood. On September 2, 1950, a biopsy of sternal marrow was taken, and the report on the microscopic examination of the sections (decalcified) was: "The sections of the bone show almost complete replacement of the medullary tissue by fibrous tissue, which is rather vascular. A few isolated cells, which appear to have come from the myeloid series, are seen. There is no question about the diagnosis of this marrow. Diagnosis: Myelofibrosis."

Course: From August, 1950, to October, 1950, the blood counts showed a decline to normal values of total white cells. The hemoglobin remained at 10 gms. In September the hemoglobin was 8 gms. on several determinations, but the total WBC declined from a range of 5,900–6,400 to 3,900–4,200. Iron studies were started August 31, 1950.

Case 4, P. N., chronic myelogenous leukemia and polycythemia vera

A 60-year-old married male, P. N., was referred in September, 1946. About two weeks before his first visit, he developed a severe ecchymosis and swelling at the left elbow subsequent to a very minor blow to the arm. He had had no radiation therapy.

Physical examination: The left elbow and forearm were swollen, slightly edematous, and ecchymotic. The spleen was firm and not tender, extending 8–10 cm. below the left costal margin on inspiration. It was estimated to be several times the normal size. The liver was not enlarged.

Laboratory examinations:

The initial red blood counts were 7.74 to 8.48 million per cu. mm.

The range in values in blood examinations during the course were as follows:

RBC	3.00–8.42 million per cu. mm.
Hemoglobin	8.3–17.7 gms. per 100 ml.
Reticulocytes	0.21–10.2%
Platelets	290,000–340,000 per cu. mm.
Hematocrit	28–63
WBC	9,200–42,000 per cu. mm.
Segmented neutrophilic granulocytes	58–88%
Band neutrophilic granulocytes	3–23%
Eosinophils	1–10%
Basophils	0.4%
Neutrophilic myelocytes	21.6%
Eosinophilic myelocytes	1.0%
Myeloblasts	0.6%
Lymphocytes	0.6%
Monocytes	0.4%
Nucleated red cells	32%

Clinical course: With some venesections, P^{32} and Y^{90}, he was maintained in good health with a less leukemoid hemogram throughout 1947 and until about mid-1948, when more myelocytes appeared. Later, promyelocytes and myeloblasts appeared, and the RBC had declined gradually so that in late 1948 and early 1949 it varied between 3 and 4 million per cu. mm., with 8 gms. of hemoglobin per 100 ml. The white counts were not lower and varied from 7,000 to 20,000 per cu. mm. The marrow exhibited a shift to more immature myeloid forms.

During 1949 the liver enlarged and the spleen increased in size, and he was given a transfusion. His blood picture now suggested the late anemic phase sometimes seen in polycythemia vera. The marrow aspiration was unsatisfactory because of dilution with peripheral blood.

He became gradually worse, and his strength and endurance diminished. By May of 1950 he noted some spontaneous ecchymoses similar to those at the onset of his illness two years previously. The spleen and liver increased in size. He had intermittent anorexia and worked less steadily at his job. After each blood transfusion he felt temporarily improved.

Unfortunately the blood volume had not been determined in the polycythemic phase. When it was done, the initial picture had changed to that of an anemia. On September 30, 1949, the blood volume, using P^{32} labeled cells, was 4,453 ml. or 64.1 ml./kg. body weight. The total red cell volume was 1,425 ml., or 20.5 ml./kg. body weight. On February 10, 1950, the blood volume was 5,914 ml., or 83.9 ml./kg., and the total red cell mass was 1,656 ml., or 23.5 ml./kg. Fe^{59} studies were started May 31, 1950.

Case 5, E. S., chronic myelogenous leukemia

A 44-year-old married woman, E. S., was referred with the diagnosis of chronic myelogenous leukemia in September, 1950. An enlarged spleen, an elevated white cell count, and a sternal marrow examination led to the diagnosis of chronic myelogenous leukemia.

Physical examination: The arms showed residual discolorations from old ecchymoses. The spleen was firm, non-tender, and palpable about 2–3 cm. below the costal margin in the left anterior axillary line. No lymph node enlargement was found.

Laboratory examinations:

The ranges in values in blood examinations were as follows:

RBC	3.3–3.6 million per cu. mm.
WBC	106,000 to 141,000 per cu. mm.
Hemoglobin	7.4–8.5 gms. per 100 ml.
Platelets	215,000–420,000 per cu. mm.

The blood volume studies by the P^{32} tagged cell method were recorded as follows:

Blood volume	4,387 ml. or 72.5 ml./kg.
Total red cell volume	1,163 ml. or 19.2 ml./kg.
Plasma volume	2,895 ml. or 47.9 ml./kg.

Course: She returned to her physician with arrangements for small doses of radioactive phosphorus. Fe^{59} studies were started September 12, 1950, prior to any radiation therapy.

Case 6, C. H., multiple myeloma

A 65-year-old married male, C. H., was referred January 9, 1950. About five months before, a hard swelling arose just above the left ankle. A biopsy was done, and the microscopic examination as well as roentgenograms of bones disclosed multiple myeloma. He had lost 15 to 20 pounds in weight during the past year. At the time he was first examined here, he complained of an unremitting pain in the right elbow.

Physical examination: The skin had a slight icteric tint. He was thin and the muscles were flabby. The spleen was palpated 1–2 cm. beneath the costal margin, and a larger than usual area of splenic dullness found. Bone tenderness was found over the distal end of the right humerus and the left ulna.

Laboratory examinations:

Prior to any therapy the range in RBC per cu. mm. was 2.97 to 4.00 million.

Hemoglobin	8.4–10.9 gms. per 100 ml.
WBC	5,400–10,400
Reticulocytes	0.6–9.7%
Hematocrit	19–28

The differential white count was within the normal range. On January 9, 1950, the aspirated sternal marrow was found to have 43% nucleated red cells and 5.2% plasma cells. On September 6, 1950, the sternal marrow contained 61% nucleated red cells and 0.8% plasma cells. Fe^{59} studies were started November 16, 1950.

Only once was the test for Bence-Jones protein in the urine positive. A month later and on many subsequent tests none has been detected.

Total protein was 4.92, albumin 2.92, globulin 2.00 gms. per 100 ml. of plasma on January 11, 1950.

Icteric index	25 on July 24, 1950, and August 8, 1950
Serum bilirubin	0.4 mg. per 100 ml. blood
Fecal urobilinogen excretion	860 mg. per 24 hours

Course: Treatment with urethane and P^{32} apparently did not alter the course. He has not required transfusions.

Case 7, C. H., refractory anemia

This 72-year-old male was first discovered to have an anemia in October, 1949.

Physical examination: The liver edge was 4–6 cm. inferior to the costal margin at the end of deep inspiration. The spleen was enlarged on deep inspiration, extending 4 cm. inferior to costal margin in the mid-axillary line.

Laboratory examinations:

The blood data showed the following ranges:

RBC	1.80–3.94 million per cu. mm.
Hematocrit	19–36
Hemoglobin	7.5–12.1 gms. per 100 ml.
Platelets	260,000–500,000 per cu. mm.
Reticulocytes	0.1–1.8%
Nucleated red cells	0–1 per 100 WBC
WBC	6,600–13,000 per cu. mm.
Segmented neutrophilic granulocytes	18–66%
Band neutrophilic granulocytes	1–30%
Metamyelocytes	0–1%
Progranulocytes	0%
Myeloblasts	0%
Eosinophilic granulocytes	0–15%
Basophilic granulocytes	0–6%
Fecal urobilinogen excretion	154–299 mg. per day
Coombs test	negative

On February 9, 1950, the pathologist's report of a marrow biopsy was as follows: "The bone marrow shows a rather moderate increase of large and nucleated red cells. This slide shows essentially the same pattern that we saw in the previous bone marrow biopsy. Diagnosis: Hyperplasia of bone marrow."

Course: The course has not been altered except for a lower requirement of blood by transfusion following splenectomy. He has received all of the various vitamins and liver concentrates as well as crude liver parenteral injections and raw liver by mouth. Fe^{59} studies started June 21, 1950, several months before the splenectomy.

RESULTS AND DISCUSSION

The plan of this part of the paper is to present the data with the discussion in the chronological manner in which the data were collected. This form allows the introductory presentation of the results of the most simple technique and progression to the more complex. The results and discussion are organized in three general sections. Section I includes the raw data of gross body surface counting rates as a function of time after a single intravenous injection of Fe^{59} during the first day of each study. It illustrates what may be learned by this technique during a relatively short study with little equipment and effort. Section II concerns a more extensive and laborious examination of the patient, the purpose of which is to learn the general location in the body of the tracer once it has left the blood stream. Section III gives data from studies which cover a two-week period. The data of the latter section are treated in a special manner by which the gross body surface counting rates are corrected for the contribution of radioactivity from plasma or red cells, and thus these data represent more nearly the tissue body surface counting rates.

I. The gross body surface counting rates over sacrum, liver, spleen and precordium during the first day after injection of Fe^{59}

These data are illustrated in Figure 4, refractory anemia with hypoplastic marrow (Case 1); polycythemia vera with myelofibrosis (Case 2); myeloid metaplasia with myelofibrosis (Case 3); chronic myelogenous leukemia (Case 5); and normal male adult.

Deviations from the normal gross body surface counting rates may be evident in some cases during the first 15 minutes after the injection of the tracer iron. As the four positions are "counted," excessively rapid disappearance of the tracer from the blood or an unusually rapid rise in counting rate over marrow, spleen, or liver may be seen directly on the moving recorder paper. A rapid and excessive rise in counting rate over liver or spleen is accompanied by a partial or complete failure of the marrow counting rate to rise (Figure 4). An estimate of the ability of the bone marrow and other tissues to accumulate radioiron is possible in the first few hours of a study. In the normal subject the sacral position was the only position showing a rising counting rate during the first eight hours. The counting rate over liver was practically constant with time, while that over the precordium and spleen fell to a near plateau level relatively soon. Since intravascular mixing time is short compared to the usual blood removal time of radioiron, it is reasonable to assume that gross body surface counting rates over a tissue during the first few minutes represent the radioactivity which is contained in the perfusing plasma. Similarly, in ten to 14 days, 80–100% of the radioactivity is again contained in blood but at this time in the red cells. Thus, in these very early periods and in the late periods, nearly all of the injected dose is in the blood, first in the plasma and later in the red cells. The contribution of blood radioactivity to the gross body surface counting rates is nearly the same at these two times except possibly in such tissues as may have different hematocrits. Thus a gross body surface counting rate constant with time during a period when the radioactivity in blood is known to be decreasing indicates that the tissue is accumulating radioactivity. This is the situation which is usually found over the liver, in contrast to the spleen where the counting rate falls as a function of time.

In contrast to the sacral counting rates of normal individuals, which rise with time, nearly constant sacral counting rates are seen in the cases represented in Figure 4. Case 3 (Figure 4), myelofibrosis with myeloid metaplasia, shows a decrease in sacral counting rate with time. This is the type of curve which might be expected in the absence of marrow erythropoiesis. Reference to the case descriptions shows the coincidence of a low counting rate over sacrum with the marrow biopsy findings of hypoplasia or aplasia of the erythroid series, or of fibrosis.

The extraordinarily high rise of counting rates occurring over spleen, as demonstrated in Figure 4 (Case 2, polycythemia vera with myelofibrosis; and Case 3, myelofibrosis with myeloid metaplasia), was not necessarily expected although there was splenomegaly in both cases. This similarity in the two cases is in contrast to the finding of polycythemia in the one and anemia in the other. The spleen counting rate of Case 5 (myelogenous leukemia), Figure 4, is intermediate and represents some ability of the spleen to accumulate iron. That

splenomegaly is not always associated with splenic removal of radioiron from plasma was demonstrated in a case having chronic lymphatic leukemia whose spleen filled half the abdominal cavity. This patient showed a spleen curve which was very similar to the normal, with no evidence of direct clearance of radioiron from plasma.

Experimentally a spleen Fe^{59} curve resembling that of the typical Fe^{59} marrow curve has been demonstrated in rats receiving total body X-radiation with their spleens protected. Direct splenic removal of radioiron from plasma was shown to be associated with the subsequent appearance of tagged red cells (9).

Figure 4 (Case 1, refractory anemia with hypoplastic marrow) demonstrates the situation wherein the usual marrow ability to deplete the plasma of tracer is replaced by a very slow removal by liver.

II. The torso survey for Fe^{59} at a time when the blood level of radioiron is minimal (Illustrated by Figure 5)

The desirability of knowing more accurately the tissue distribution of tracer when it was nearly absent from blood led to the system of body profile counting rate and its associated polar plot, described earlier in this paper in METHODS under "Torso Survey at the Time of Minimal Blood Tracer Concentration." The blood tracer concentration in each case was ascertained to be minimal by reference to data, as shown in Figure 3. The averaged data of five normal male adults are graphed in Figure 5. The shape of the uppermost levels with the posterior projection indicates the association of vertebrae with the iron isotope. The subsequent finding of the tracer incorporated in the red cells is an indication of a normally functioning marrow.

In the third and fourth levels of the plots of the normal cases the shape is similar. There is a slight asymmetry with a right preponderance, which probably indicates the normal liver iron turnover. That the third and fourth levels are not more asymmetrical shows that the major stream of iron from plasma is normally to bone marrow. Finally, the larger area of the lowermost level shows that this region in the 23–33 year age group probably contains a considerable portion of the red marrow of the body. That this distribution may not hold for all age groups is indicated by the finding of a contracted sixth level in a 72-year-old normal male.

In the early studies it was thought worthwhile to explore the head and the extremities as well as the torso; however, the counting rates of the skull and the extremities were usually very low. It was noted that excessive erythroid marrow hyperplasia was associated with a more diffuse skeletal distribution of radioiron, resulting in contracted polar plots of the torso (Figure 5, Case 6, multiple myeloma with hemolytic anemia).

The grotesque figures (Figure 5) from Case 1, refractory anemia with hypoplastic marrow; Case 2, polycythemia vera with myelofibrosis; Case 3, myeloid metaplasia with myelofibrosis; Case 4, chronic myelogenous leukemia following polycythemia vera; and Case 5, chronic myelogenous leukemia, exemplify the aberrant iron kinetics which are associated with certain abnormalities in hematopoiesis.

Case 1, Figure 5 (refractory anemia with hypoplastic marrow, the same patient as shown in Figure 4) demonstrates the relative failure of tracer to accumulate in marrow areas and negligibly in spleen. The fraction of tracer appearing in red cells in this case never exceeded 0.12 of the injected dose, and the liver counting rate increased slowly with time and never decreased.

Case 2, Figure 5 (polycythemia vera with myelofibrosis, the same as in Figure 4) shows the major accumulation of tracer in the splenic region with very little in the normal marrow areas. That this abnormal location of iron was associated with red cell production in the spleen was indicated by the high fraction of the tracer dose which appeared in red cells and is confirmed by the polycythemia and the morphologic finding of myelofibrosis.

Case 3, Figure 5 (myeloid metaplasia with myelofibrosis) shows a large portion of the tracer in spleen and also a significant amount in liver. A bone marrow biopsy from this patient was reported as showing almost complete replacement of the normal medullary tissue by fibrous tissue. The maximum fraction of the tracer which was present in the erythrocytes at any one time during the first two weeks of study was 0.6. The concomitant drop in counting rates over spleen and liver with a rise in erythrocyte concentration of tracer indicated the possibility that the liver as well as the

spleen was involved in erythropoiesis. The asymmetry of the polar plot with a left preponderance, indicated that the spleen was the more important for this purpose. This pattern differs from myelocytic leukemia (Case 5, Figure 5) in which evidence of hepatic and splenic metaplasia as well as some marrow function exists.

Case 4, Figure 5 (chronic myelogenous leukemia and polycythemia vera) shows a lack of the expected gross body surface counting rates in the normal marrow locations. Instead, the tracer rapidly accumulated almost equally between the liver and the spleen. The subsequent appearance of approximately 0.4 of the tracer dose in red cells, with a concomitant fall in counting rate over the spleen and a constant counting rate over liver, showed erythroid metaplasia of the spleen and suggested storage function of the liver. Repeated marrow aspirations during the course of the illness indicated a diminishing marrow cellularity.

The previous discussion of results is illustrative of the usefulness of the application of *in vivo* Fe^{59} methods in following the immediate movement of the tracer from plasma to the various tissue sites.

III. The tissue body surface counting rates over sacrum, liver, and spleen during the first two weeks after the injection of Fe^{59} (Illustrated by Figures 6–8)

A more comprehensive insight is gained of the functional significance of the particular tissue distribution if plasma and red cell sampling as well as body surface counting rates are continued for a longer period of time. The data presented in Figures 6–8 are the gross body surface counting rates per microcurie injected minus the counting rate contributed by blood. The curve illustrated in Figure 3 was used for this correction (see METHODS).

Figure 6 shows such data from the representative marrow site. Included are three cases: a normal male adult, a patient with refractory anemia, hyperplastic marrow (Case 7), and a patient with refractory anemia, hypoplastic marrow (Case 1). The curve of the representative marrow site from the normal subject, in its time concentration relationship, resembles very closely similar data which have been obtained by serial animal tissue sampling (9, 10). Comparison of the data of the

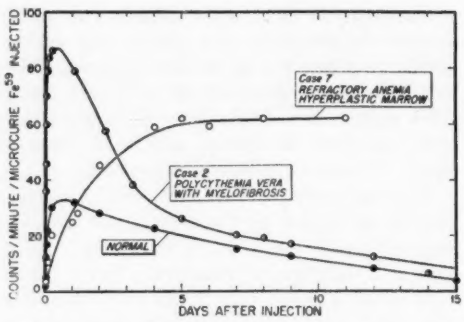

FIG. 7. TISSUE BODY SURFACE COUNTING RATES OVER SPLEEN AS A FUNCTION OF TIME AFTER A SINGLE INTRAVENOUS INJECTION OF Fe^{59}

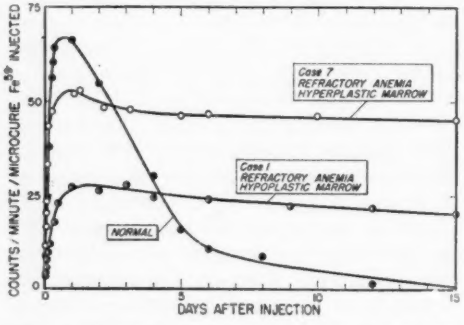

FIG. 6. TISSUE BODY SURFACE COUNTING RATES OVER SACRUM AS A FUNCTION OF TIME AFTER A SINGLE INTRAVENOUS INJECTION OF Fe^{59}

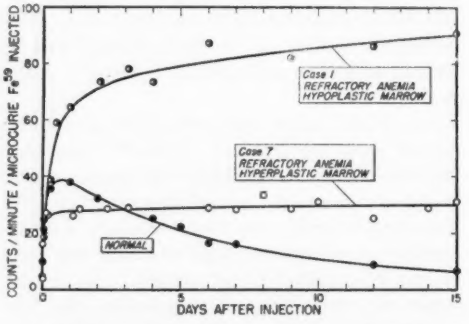

FIG. 8. TISSUE BODY SURFACE COUNTING RATES OVER LIVER AS A FUNCTION OF TIME AFTER A SINGLE INTRAVENOUS INJECTION OF Fe^{59}

normal subject of Figure 6 with Figure 3 shows the concomitant rise in red cell concentration of tracer and the fall in marrow counting rate. It is not surprising that the counting rate over the marrow site of Case 7 (refractory anemia, hyperplastic marrow), Figure 6, rose to a fairly high level, almost normal. That it did not rise as high as the normal is possibly associated with the greater dispersion of functioning marrow. Corroborative evidence was the finding of slightly contracted polar plots, as with Case 6, Figure 5. Also, it was expected that the marrow of the hypoplastic anemia patient (Case 1) would accumulate very little Fe^{59}. The surprising point was the failure of the subnormal counting rates of Cases 1 and 7, Figure 6, to fall. This phenomenon need not be considered a static one; *i.e.*, it is not necessarily indicative of iron accumulation in the marrow without discharge. Instead, it may indeed indicate a very rapid turnover through marrow with a high equilibrium level. Indirect evidence that the latter is probably true is gained from other considerations of these and other cases. For example, such findings are usually associated with a "secondary" rise in spleen counting rates, as illustrated by Case 7 in Figure 7 (refractory anemia with hyperplastic marrow). This rise is secondary in that it does not occur as the iron is initially cleared from the plasma but only subsequent to its appearance in marrow. Case 6, having multiple myeloma and a severe anemia, had data of this nature and had additional evidence of extraordinary erythropoietic hyperfunction in that the marrow showed 65% of the cells to be of the erythroid series and the stool urobilinogen excretion to be greater than 800 mg. per day. On the other hand, there have been observations showing that patients such as Case 7 may have hemosiderin granules in their marrow. Thus, a possible reason for the marrow counting rate not being near zero two weeks after injection in Case 1 may be related to non-erythrogenic medullary iron function (11).

From Figure 7 a comparison may be made of tissue body surface counting rates over the spleen in a normal subject and in two patients. The spleen curve of Case 2 (polycythemia vera with myelofibrosis) is remarkably similar in time concentration relationship to the normal marrow curve (Figure 6). The drop in counting rate over the spleen in Case 2 was associated with a rise in the erythrocyte tracer concentration to near normal levels. *This type of spleen curve has been called erythrogenic in that it rises as the concentration of the injected radioiron in plasma falls, and falls as the erythrocyte concentration of tracer rises.* Another type of curve associated with spleen, distinctly different from the primary type, is illustrated by the data of Case 7 (refractory anemia with hyperplastic marrow), Figure 7. In this instance a great *rise in counting rate over spleen occurs following or simultaneously with a fall in counting rate over sacrum and concomitantly with a subnormal rise in erythrocyte concentration of radioiron. This type of splenic radioiron curve has been called erythroclastic.* Despite the fact that these tissue body surface counting rates over spleen are corrected for the contribution of counting rate by radioisotope in the circulating blood (see METHODS), they are higher at three to 10 days than they are when all of the isotope is in the plasma; *i.e.*, just after injection.

Convincing evidence that the erythrogenic spleen curve, as seen in Case 2 (polycythemia vera with myelofibrosis), is the result of splenic erythroid metaplasia are the facts that this patient (see Case 2 report) had myelofibrosis and polycythemia (red cell mass, 54.6 ml./kg.), and that nearly all of the tracer was in red cells within 10 days after the dose was given. The polar plots (Figure 5, Case 2) suggest the presence of myelofibrosis throughout the areas usually containing normal marrow.

Corroborative data showing that the erythroclastic spleen curve is associated with excessive cell destruction are given in the case reports of Cases 6 and 7, which had this type of spleen curve. The pertinent findings are anemia, marrow hyperplasia, reticulocytosis, high iron turnover rates, near normal uptakes of radioiron in marrow, high stool urobilinogen excretion, and a high icteric index. The extirpated spleen of Case 7 (refractory anemia with hyperplastic marrow) showed no evidence of erythroid metaplasia but did show hyperplasia of the reticulum. There was no evidence of excessive deposits of iron pigments in the spleen. It weighed 585 gms. The routine findings in these two individuals (Cases 6 and 7) which are ordinarily associated with the diagnosis of hemolytic anemia are different in that they are

clear-cut and definite in Case 6 but questionable or absent in Case 7. In contrast, plasma iron turnover rates are increased in both cases; and both cases show the *erythroclastic curve*.

We believe this affords a method for distinguishing some anemias of abnormal formation from those of abnormal destruction. The findings of a more rapid than normal iron turnover, *erythroclastic curve*, the absence of excessive urobilinogen excretion, and the absence of high serum bilirubin and reticulocytes go together to indicate an abnormality or failure in the formation of hemoglobin and the rapid destruction of abnormal cells. This type of case could well be called an anemia of abnormal hemoglobin formation with resulting short cell life (12).

The second type of case in which the usual findings indicating abnormally rapid destruction and formation of erythrocytes are present with excessive iron turnover and an *erythroclastic spleen curve* could be considered as having an abnormally rapid destruction of red cells, not the result of an inherent abnormality in hemoglobin formation, but existing independently of erythrogenesis except for the stimulation it affords.

In Figure 8 is presented the tissue body surface counting rate over liver from a normal subject and from two patients with refractory anemia (Case 1 and Case 7). The curve from the patient with a hypoplastic marrow (Case 1) differs from the normal in that it rises beyond the expected value and after 12 to 14 hours continues to rise, albeit at a slower rate, while that of the normal tends to decrease slowly. The patient having the hyperplastic marrow (Case 7) shows a failure of the counting rate over liver to reach the normal value and then tends to increase slowly.

Case 1, with a hypoplastic marrow, illustrates the failure of the major pathway of iron from plasma to marrow to function and the substitute of the liver as the major pathway. The gradual slow gain in counting rate over liver is in accordance with the well-known function of the liver to effect net gains in iron when there is a failure in red cell formation or when extra iron is introduced into the body. The patient having the hyperplastic marrow (Case 7) demonstrates that the major exit of the iron from the plasma may continue to be marrow, even in the presence of severe anemia and abnormalities in medullary morphology. In fact, the diversion of iron to this type of marrow is possibly greater than normal and accounts for the failure of counting rate over the liver to rise initially to the normal value. The rising counting rates over liver in the later days of the study indicate net gains of iron. Measurements over this patient's liver were continued for 70 days, at which time the counting rate over liver was higher than over any other area.

SUMMARY

The kinetics of intravenously injected Fe^{59} Globulin IV-7 in tracer doses were observed by external scintillation counting over marrow, liver, and spleen as well as by *in vitro* analysis of plasma and erythrocyte radioactivity. Thirty patients and seven normal subjects were studied.

The quantity of tracer in the plasma and red cells was plotted as a function of time; and the relative counting rates representing the other three compartments (marrow, liver and spleen), free from the counting rate contributed by blood, were plotted in a similar manner. In order to assess the significance of a tissue counting rate, a survey of the torso was made when the combined plasma and erythrocyte level was minimal. Polar coordinate plots of the relative counting rates of six cross-sectional torso levels indicated the relation of the spleen, liver, or marrow counting rates to those over the remainder of the body.

The kinetics were remarkably uniform in young male adults. The tissue of major importance in depleting the plasma of the tracer was the bone marrow. The time required for the plasma depletion was quite uniform. This major initial transient accumulator, the bone marrow, was observed simultaneously to discharge the tracer as it appeared in the circulating erythrocytes. A minor initial transient accumulation occurred in the liver. This was seen to feed back into the major pathway: plasma, to marrow, to red cells.

In the patients, abnormalities in the initial direction or destination as well as in velocity were observed.

In subjects having known marrow hyperplasia without splenic or hepatic abnormality (for example, secondary polycythemia), the velocity from plasma to marrow was increased. The marrow volume apparently included the extremities as was

evidenced by the lesser area of the normally shaped torso polar plots.

When the major initial transient accumulator was spleen, there was always an increased velocity. Such patients showed microscopic evidence of lack of erythrogenic tissue in the bone marrow. If the initial transient major accumulation was divided among liver, marrow and spleen or liver and spleen alone, the velocity was also great.

The presence of a large spleen was found not to be indicative of a particular type of iron kinetics. Two types of splenic curves were observed which are thought to represent erythrogenic and erythroclastic phenomena. The conditions for the *erythrogenic spleen curve* were: (*1*) The spleen showed the ability to accumulate a greater than normal amount of radioiron as the high initial plasma concentration fell; and (*2*) The spleen subsequently discharged this excess of radioiron as the red cell concentration of radioiron rose. The condition with the *erythroclastic spleen curve* was: The spleen showed the ability to accumulate an abnormally great amount of radioiron concomitantly with a fall in marrow radioiron and a rise in red cell concentration. "Erythroclastic" is not used to indicate that the primary abnormality is necessarily splenic but only to indicate excessive cell destruction.

Two cases are presented having *erythroclastic spleen curves,* one of which had findings indicating that the spleen was primarily at fault, and the other having evidence of abnormal hemoglobin and cell formation.

The liver was never observed to be the major remover of iron from the plasma to discharge it into cells; however, it, with the spleen, might share in various portions the delivering of tracer to the second major compartment, the red cells.

A subnormal velocity was associated with the exclusive hepatic removal of tracer from plasma. For example, 48 hours might be required to deplete the plasma of tracer to a level that would be achieved in one or two hours in a case where the spleen or bone marrow was the receptacle. However, exclusive hepatic removal was not always associated with plasma iron turnover rates which were markedly below normal. When the liver was the major primary accumulator, it continued to maintain high levels for many days and no secondary wave of high counting rates appeared over any of the other four compartments studied.

Failure of the normal second major compartment, the circulating erythrocytes, to exhibit accumulation of Fe^{59}, was associated with all variations in the preceding kinetics; that is, plasma to spleen, plasma to liver, or plasma to bone marrow. An interesting partial failure of the erythrocytes to accumulate tracer even though the marrow depleted the plasma or Fe^{59} occurred with the appearance of a third receptable, the spleen. In one of these patients who presented the usual findings of excessive hemoglobin production and destruction, the velocity of Fe^{59} from plasma to marrow was great, and there was no other tissue collecting the tracer. The marrow appeared to empty itself only partially of the tracer; moreover, the erythrocytes did not accumulate their normal portion, and simultaneously splenic counting rates rose over a two to four day period. Finally, an abnormal equilibrium was established in which the levels in spleen and marrow were very high, while that of the erythrocytes was unusually low.

It is concluded that *in vivo* studies with Fe^{59} are of value in the study and diagnosis of various hematopoietic disorders. These studies are also a useful adjunct in the guidance of the clinician in therapy.

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THE EFFECT OF ADRENOCORTICOTROPHIC HORMONE (ACTH) ON CEREBRAL BLOOD FLOW AND METABOLISM [1]

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While patients receiving adrenocorticotrophic hormone (ACTH) often show an increased sense of well-being and mild euphoria, a small number become psychotic. Electroencephalographic (EEG) changes (1) have been reported in patients receiving ACTH who become psychotic, but the changes have been inconsistently present. In one instance (2) the EEG changes appeared to be related to a decrease in serum potassium level.

These observations led to a study of cerebral blood flow and metabolism in patients receiving ACTH. Concurrent investigation of the EEG (3) and of the clinical and metabolic responses of these subjects will be reported elsewhere (4).

METHOD

A total of 44 observations on 14 patients was made; 15 before, 23 during, and six following ACTH treatment. ACTH was administered intramuscularly to adults in full therapeutic doses (60–100 mgs. Armour ACTH or 30 to 40 mgs. Wilson's Corticotropin daily).[3] One 12 year old child received 33 mgs. Armour ACTH daily. There was no significant difference noted in the clinical response to the two brands of ACTH at these doses. Measurements were made at least once during therapy and as often as four times at intervals varying from seven to 56 days. In all patients, except one studied at seven days, at least one of the repeat studies was made between the second and third week on therapy. Several disease states are represented, including five patients with active rheumatoid arthritis, three with disseminated lupus erythematosis, and one each with subacute glomerulonephritis with edema, chronic glomerulonephritis, primary systemic amyloidosis, myasthenia gravis, chronic pulmonary fibrosis, and chronic exfoliative dermatitis. All patients showed increased appetite and sense of well-being and most developed the facial fullness and redness suggestive of Cushing's syndrome.

All of the patients were studied under similar laboratory conditions in the fasting state. The nitrous oxide method originally described by Kety and Schmidt (5) and modified by Scheinberg and Stead (6) was used. The gas mixture used in the determination consisted of approximately 15% N_2O, 64% N_2, and 21% O_2. Arterial blood pressure was measured by the auscultatory method with the arm held at heart level. Mean arterial blood pressures were calculated from these readings by adding one third of the pulse pressure to the diastolic pressure. Blood samples for determination of oxygen and glucose contents were drawn just before and following each cerebral blood flow. The A-V oxygen difference was determined by the method described by Hickam and Frayser (7). The blood glucose content was measured by Nelson's (8) photometric adaptation of the Somogyi method. Cerebral oxygen and glucose utilizations were calculated as previously described (5, 6).

RESULTS

The results summarized in Table I show a statistically significant decrease in cerebral blood flow on ACTH treatment from 61 to 50 ml./min./100 gm. brain. After approximately 14 days on therapy no further decrease in cerebral blood flow oc-

TABLE I

Effect of ACTH on cerebral blood flow and metabolism
(Mean values of 44 determinations on 14 subjects)

	No ACTH (21 Determ.)	On ACTH (23 Determ.)	P-Value
CBF (ml./min./100 gm. brain)	61 ±3.2	50.0±2.0	0.01
A-V O_2 Diff. (vols. %)	5.8± .22	6.5± .23	0.05
A-V Gl. Diff. (mgs. %)	8.2± .50	10.1± .45	0.02
CMR O_2 (ml. O_2/min./100 gm. brain)	3.5± .50	3.2± .33	Not significant
CMR Gl. (mg. glucose/min./100 gm. brain)	4.9± .40	4.9± .24	Not significant
MABP (mm. Hg)	90 ±2.0	98 ±2.7	0.05 to 0.01
CVR (units)	1.6± .12	2.1± .14	0.05 to 0.01

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[2] American Heart Association Research Fellow.

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curred in spite of continued full ACTH dosage. The arterial-cerebral venous oxygen and glucose differences were increased and hence there was no demonstrable change in cerebral utilization of oxygen and glucose (CMR O_2 and CMR Gl.). During ACTH therapy a 9% increase in mean arterial blood pressure (MABP) occurred. There was, however, a 32% increase in cerebral vascular resistance (CVR) since the decrease in cerebral blood flow was greater than the rise in mean arterial blood pressure.

Six patients with normal or high pre-treatment cerebral blood flows had studies performed both before initiation and after discontinuation of ACTH therapy. In this group there was only a 3 ml./min./100 gm. brain difference and thus the before and after flows have been grouped together in the column labeled "No ACTH" in Table I.

There were six patients included in Table I whose cerebral blood flow before ACTH therapy was below 50 ml./min./100 gm. brain. These patients also showed low oxygen and glucose consumption by the brain. In these six patients ACTH treatment was not associated with further reduction in cerebral blood flow (46 to 45 ml./min./100 gm. brain), nor was oxygen consumption (2.8 to 2.9 ml. O_2/min./100 gm. brain) significantly altered. Although these patients did develop a similar slight hypertension as did the group with normal initial flows, no significant change in cerebral vascular resistance occurred. The clinical response to ACTH treatment was similar regardless of the original level of cerebral blood flow.

Two patients were studied before, during and following a psychotic episode which developed during ACTH therapy. These studies were not different from the cerebral blood flow changes which occurred in other patients receiving ACTH who did not become psychotic. Thus psychotic behavior during ACTH therapy was not associated with change in cerebral metabolism as measured by this method. Serial EEG studies were done on all patients (3). In 12 there was no gross change in the EEG pattern, including the two patients who had tracings during their period of psychosis. Definite improvement in EEG pattern was found on therapy in two patients. There appeared to be no correlation between changes in cerebral blood flow and EEG patterns. Nor was it possible to correlate changes in plasma carbon dioxide combining power or serum potassium levels with changes in either cerebral blood flow or mental status.

DISCUSSION

The results show ACTH significantly decreased cerebral blood flow without changing cerebral oxygen and glucose consumption. This response occurs before or by the end of the second week of therapy, and seems to persist unchanged as long as the therapy is continued. The effect is reversible in that cerebral blood flow returns to pre-treatment levels within a few days after discontinuation of treatment with ACTH. In patients with abnormally low pre-treatment cerebral blood flow ACTH did not change either cerebral blood flow or cerebral metabolism.

How ACTH decreases cerebral blood flow is unknown. The decrease in cerebral blood flow could be related to generalized increase in peripheral resistance, to a decrease in cardiac output, or to a localized increase in resistance in the cerebral circulation. The rise in arterial pressure is less than would be expected if the peripheral resistance as a whole had increased to the same extent as the resistance in the cerebral vessels. In fragmentary preliminary studies, ACTH has not consistently changed cardiac output (9–11). Horwitz and colleagues have reported that the cardiac output frequently decreases in those instances in which ACTH produces a fall in heart rate. The best explanation from the available data is a local increase in cerebral vascular resistance of unknown etiology.

A reduction in cerebral blood flow out of proportion to the general increase in peripheral resistance has been observed in subjects breathing 85% oxygen and during hyperventilation (12). The cerebral metabolism of oxygen and glucose is normally maintained by simultaneous and adequate widening of their respective A-V differences.

It is well known that cerebral vessels are extremely sensitive to changes in blood pH (12) and carbon dioxide content. In respiratory alkalosis a decrease in cerebral blood flow does occur. In a few patients with Cushing's disease and occasionally in those receiving ACTH metabolic alkalosis develops. The production of metabolic alkalosis in humans with 3% sodium bicarbonate intrave-

nously (13) has been found to increase cerebral blood flow markedly without changing cerebral metabolic rate. Thus it seems unlikely that metabolic alkalosis was responsible for the decrease in cerebral blood flow. It is known that hypothyroidism develops on prolonged ACTH treatment (14). The patients in this study, however, did not have any decrease in cerebral metabolic rate of oxygen consumption which characterized those patients studied by Scheinberg (15) with hypothyroidism.

SUMMARY AND CONCLUSIONS

1. Studies of cerebral blood flow and metabolism were made on 14 patients receiving ACTH for various diseases.

2. An 18% decrease in cerebral blood flow occurred during ACTH therapy. This was associated with a 32% increase in cerebral vascular resistance and 9% increase in mean arterial blood pressure. These changes were accompanied by significant widening of arterial-cerebral venous oxygen and glucose differences, and hence no change in cerebral metabolic utilization of oxygen or glucose.

3. The decrease in cerebral blood flow during ACTH treatment occurs before or by the end of the second week, apparently persists unchanged on continued therapy, and returns to pre-treatment levels within several days after discontinuing ACTH.

4. No information was found by this method to explain either the sense of well-being or the psychotic episodes which occur during ACTH therapy.

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TREATMENT OF NEPHROSIS WITH PITUITARY ADRENOCORTICOTROPHIN [1]

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INTRODUCTION

The administration of pituitary adrenocorticotrophin (ACTH) to patients with nephrosis has been reported to initiate diuresis in a large proportion of cases (1–6). Since this diuresis may occur either during or after the administration of ACTH, it is not clear how such a diuresis is related to the secretions of the adrenal cortex. Renal function is usually markedly improved (7), but the relationship in time of such improvement with diuresis is not definitely established. In order to visualize the timing of some changes in renal and adrenal function, together with the shifts in fluids, electrolytes, and proteins, a continuous series of measurements has been made during treatment of nephrosis with ACTH.

SUBJECTS AND METHODS

Fourteen patients with nephrosis have been treated with ACTH. Patients varied from two to 50 years of age. All presented the edema, proteinuria, hypoproteinemia, and hypercholesterolemia characteristic of the syndrome. No patient had a systolic blood pressure above 145 mm. Hg before treatment. Only one patient had an elevated serum creatinine level (3.0 mg.% in case 12). Quantitative examination of the urinary sediment showed no abnormality in cases 3, 7, and 8; the presence of some casts and epithelial cells containing fat, and 1 to 5 million red blood cells per 24 hours in cases 1, 2, 4, 5, 10, 11, and 12; and a heavy sediment containing many casts, epithelial cells loaded with fat, and 15 to 120 million red blood cells per 24 hours in cases 6, 9, 13, and 14. In case 9, the nephrotic syndrome was associated with signs of disseminated lupus erythematosus. In case 11, there was a severe hemolytic anemia which responded fairly well to cortisone and remained relatively inactive during treatment with ACTH. In cases 3, 6, 7, 11, and 14, cortisone had been administered without a satisfactory diuresis.

Controls, experimental procedure, and follow-up have been described in a previous report (8). Total serum protein and albumin were determined by micro-Kjeldahl analysis for nitrogen. The separation of albumin with sodium sulfate, used in the present work, has been previously (9) found to give a value for serum albumin which is approximately 0.7 gm./100 cc. greater than that obtained by electrophoresis. Because of the error introduced in the electrophoretic technique by the obvious variations in lipemia, the salting-out method was selected as more appropriate for comparing results before and after treatment. Protein in urine was measured in Shevsky-Stafford tubes after centrifugation in a constant-speed centrifuge and corrected for the small variations in temperature.

"Creatinine" was determined without preliminary separation and thus includes other chromogens. Since multiple daily venepunctures were avoided in order to reduce blood loss, the effect of possible diurnal variation in the plasma "creatinine" was not taken into account except when short-term clearances were measured.

ACTH was obtained from Armour and Co. as a lyophilized powder. Daily dosage was given by intramuscular injection in four equal parts at intervals of six hours. Several different lots of ACTH were used in the course of the study, but there was no evidence that failure was due to absence of adrenal cortical response, with the possible exception of case 12, in which neither a satisfactory eosinopenia nor a significant rise in 17-ketosteroids was observed during administration of ACTH.

RESULTS

The effects of administration of ACTH on diuresis and on edema varied in degree and in time of appearance (Tables I and II). Four general types of response were noted:

Group 1. Diuresis with complete elimination of edema during administration of ACTH.

Group 2. Diuresis beginning during administration of ACTH, completed after end of therapy.

Group 3. Diuresis beginning after discontinuing administration of ACTH.

Group 4. No effective diuresis.

Although these differences in time of diuresis are not clearly related to the duration of treatment, it is possible that all patients would have eliminated their edema if treatment had been continued for

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a longer period. Patients in groups 3 and 4, however, showed no signs of such improvement during nine to 14 days of treatment.

Group 1. Two patients, one with heavy edema and one with slight edema, showed oliguria and reduced sodium excretion for the first three days of ACTH administration. Subsequently increasing diuresis and sodium excretion resulted in complete elimination of edema by the end of treatment on the 11th and 13th days (Figure 1, Tables I and II).

Group 2. Four patients, two of whom received a second course of treatment with similar results, showed more or less oliguria for the first one to five days of ACTH administration (Table II). The depression of urine volume occurred even when sodium excretion before treatment was too low to be significantly reduced by ACTH. Subsequent diuresis reached a maximum on the fifth to tenth day of treatment. In two cases, diuresis and elimination of edema at the end of ACTH administration were proceeding at a steady or increasing rate, which increased sharply when ACTH was withheld (Figure 2). In these cases, diuresis might well have gone on to complete relief of edema if treatment had been continued. In the other two cases, however, the rate of diuresis had reached a maximum and was falling at the end of ACTH administration, leaving considerable edema. A second peak of weight loss and of sodium excretion appeared on the first to fourth day after the end of therapy (Figure 3).

Group 3. Four patients showed oliguria and sodium retention, as in the other patients, but effective diuresis did not occur during the second week of treatment (Table II). The serum sodium concentration remained low. Sodium excretion

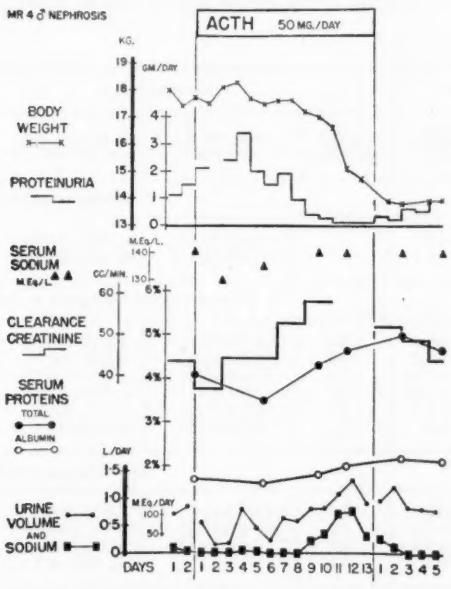

FIG. 1. DIURESIS WITH ELIMINATION OF EDEMA AND REDUCTION OF PROTEINURIA TO NORMAL DURING ADMINISTRATION OF ACTH (CASE 2)

Aggravation of all abnormalities during first few days of treatment was followed by improvement during the second week on ACTH. There was a rapid return toward control levels when treatment was stopped. Edema reappeared one month later.

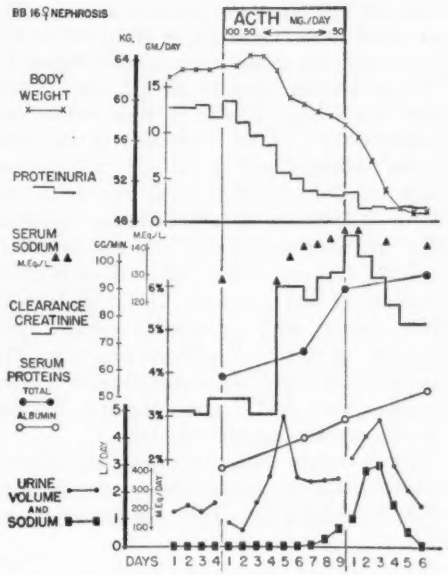

FIG. 2. DIURESIS DURING AND AFTER ADMINISTRATION OF ACTH (CASE 3a)

After the fourth day of ACTH, there were sharp increases in creatinine clearance and in urine volume, followed by rising serum sodium and finally by a rise in urinary sodium. Diuresis increased when ACTH was withheld. Proteinuria remained low after treatment. Serum proteins reached a peak of 6.3% (albumin 3.9%) two weeks after treatment.

remained well below the intake of sodium. Observations at the end of nine to 14 days of ACTH gave no promise of impending diuresis (Figure 4).

When administration of ACTH was stopped, there was a large increase in urine volume, beginning within 24 hours of the last injection. The serum sodium concentration rose rapidly, and large amounts of sodium were excreted in the urine. Elimination of edema followed in five to nine days after the end of ACTH administration.

Group 4. Four patients had no effective diuresis during or after treatment. This group included three patients with considerably reduced renal function due to chronic glomerulonephritis. The fourth patient (case 13) developed signs of acute infection when ACTH was withdrawn, followed by delayed and sluggish diuresis.

Serum sodium concentration was frequently below the average normal level before administration of ACTH (Table II). There was no obvious relationship between the degree of restriction of sodium intake and the individual levels of serum sodium before treatment. When ACTH was administered, the serum sodium concentration usually fell (average − 5 m.eq./L) with the initial reduction of urine volume and gain in weight (Table II, Figures 1 and 4). Restriction of sodium intake may have exaggerated the fall in sodium concentration. After ten days of treatment, the serum sodium concentration was 140 m.eq./L or higher in nine of the 16 observations. Groups 1 and 2 were consistently normal (average 142 m.eq./L, Table II) at the end of treatment. In group 3, the concentration of sodium fell to very low levels (Table II) and was still far below normal (average 128 m.eq./L) at the end of treatment. Group 4 showed a variable pattern. No specific symptoms referable to the lowered serum sodium concentration were noted.

The fall in the serum sodium concentration in

TABLE I

Case No.	Age (yrs.)	Duration of edema (months)	ACTH		Body weight		Proteinuria*		
			Days Rx	Total (mg.)	After Rx (kg.)	Weight change (kg.)	Control average (gm./day)	Range on Rx (gm./day)	Range post Rx (gm./day)
									I. Diuresis complete
1	4	3	13	650	13.7	−4.1	1.1	3.4(4)–0.08(11)	0.21(2)–1.08(7)
2	7	2	11	550	27.6	−2.4	2.4	3.0(3)–0.08(10)	0.12(2)–0.07(6)
									II. Diuresis started during treatment,
3a	16	2	9	500	48.9	−14.3	12.5	13.4(1)–2.9(9)	3.3(1)–1.0(26)
3b	16	8	10	600	55.0	−4.1	6.0	11.5(2)–0.3(10)	0.5(2)–0.1(7)
4	3	24	10	800	15.7	−2.9	1.9	1.6(1)–0.4(10)	1.1(1)–0.2(2)
5a	2	5	13	650	13.8	−1.0	1.6	1.3(7)–5.0(13)	4.8(8†)–0.2(20)
5b	3	10	12	1,030	13.1	−3.3	2.7	5.4(4)–0.7(11)	0.5(3)–0.2(6)
6	22	11	10	500	63.4	−6.1	14.7	15.0(1)–29.2(6)	23.0(3)–15.2(5)
									III. Diuresis prima-
7	50	4	9	550	49.5	−10.5	3.3	6.1(6)–3.1(8)	1.2(3)–2.5‡(7)
8	2	8	11	550	11.1	−5.6	1.6	— −0.11(10)	0.11(1)–0.02(8)
9	39	6	14	1,500	55.8	−4.6	6.0	7.1(3)–16.0(13)	10.6(3)–4.7(11)
10	3	4	10	250	16.2	−7.3	5.5	3.2(3)–9.1(7)	10.3(5†)–1.5(11)
									IV. Diuresis
11	34		10	1,000	51.1	−0.4	6.2	8.9(3)–4.3(8)	4.4(1)–6.3(4)
12	39	36	10	525	97.7	−1.8	11.5	Unchanged	Unchanged
13	3	7							
14	35	2	12	640	78.1	−1.4	16.0	13.7(1)–21.3(12)	23.2(5)–16.4(8)

* Number in parenthesis indicates day of treatment or after treatment when maximum or minimum was observed.
† Intercurrent infection.

the early days of treatment was usually associated with increases in body weight and edema and with a fall in urine volume, suggesting a dilution of the extracellular fluid. The return of the serum sodium concentration to higher levels with increasing urine volume at the beginning of diuresis supported this impression. However, some obvious discrepancies suggest that other factors play a role. Exchanges of sodium between cells and extracellular fluid are by no means excluded.

Increased excretion of sodium in the urine appeared at a variable level of serum sodium, generally approaching normal levels (Figures 1–4).

Endogenous "creatinine" clearance increased regularly after the first few days of treatment (Table II). The average increase was 63% of the control level. Improvement often continued for some days after administration of ACTH was stopped. Then there was usually a gradual decline in clearance, but case 3 (Table II) still showed a virtually normal "creatinine" clearance when she returned six months after the first course of treatment because of recurrence of edema.

No consistent difference in the changes in "creatinine" clearance between the four groups was evident to account for the difference in time of diuresis.

The relationship between the clearances of "creatinine" and inulin has not been systematically measured. A few observations (Table IV) indicated that the general pattern of changes in the two clearances could be parallel. Barnett and his co-workers (7), using a more specific creatinine method, have noted much greater rises in inulin clearance than in creatinine clearance in children with nephrosis. In some of the cases reported here, the changes in glomerular filtration rate may thus have been even greater than the observed changes in "creatinine" clearance.

Sodium-retaining activity of the corticoid fraction of urine was equivalent to 3.4 to 9.6 γ of desoxycorticosterone per 20 minutes of urine

TABLE I (*Continued*)

Serum proteins			Serum albumin			Serum cholesterol			Case No.
Control (*gm./100 cc.*)	End B (*gm./100 cc.*)	Maximum (*gm./100 cc.*)	Control (*gm./100 cc.*)	End B (*gm./100 cc.*)	Maximum (*gm./100 cc.*)	Control (*mg./100 cc.*)	End B (*mg./100 cc.*)	Minimum (*mg./100 cc.*)	
during treatment									
4.1	4.6	5.0	1.7	2.0	2.2	770	—	485	1
4.2	5.7	5.7	2.0	3.6	3.8	980	680	620	2
completed after end of treatment									
3.9	6.2	6.3	1.8	2.9	3.9	607	877	550	3a
4.5	6.1	6.7	2.2	3.4	4.6	634	448	280	3b
3.4	4.1	5.2	1.3	2.0	2.7	737	680	514	4
3.7	4.6	4.4	1.5	2.0	2.0	910	—	725	5a
3.5	4.7	5.9	1.7	2.6	3.0	1120	850	542	5b
3.7	4.3	5.0	1.8	2.0	2.4	1100	877	1020	6
rily post-treatment									
4.0	4.1	5.0‡	1.6	2.0	2.7‡	670	607	412	7
3.1	2.7	4.1	0.5	1.2	2.0	—	725	390	8
5.6	4.9	5.6	2.4	2.2	2.4	300	417	370	9
3.9	4.6	4.6	1.2	2.5	2.5	1100	980	530	10
ineffectual									
4.0	4.6	4.2	1.9	2.8	2.0	440	—	455	11
2.9	3.3	3.2	1.7	1.8	1.8	—	—	—	12
4.4			1.5						13
3.6	3.6	3.6	1.2	1.6	2.1	1100	1070	804	14

‡ Follow-up six weeks after discharge: Proteinuria 0.1 gm./day; serum proteins 6.0, albumin 4.2 gm./100 cc.

TABLE II

Case No.	Edema		Sodium intake (m.eq./day)	Sodium output in urine				Urine	
	Control	Minimum post B		Control average (m.eq./day)	During treatment		After B maximum (m.eq./day)	Control average (cc./day)	During
					Minimum (m.eq./day)	Maximum (m.eq./day)			Minimum (cc./day)
									I. Diuresis complete
1	4+	0	17.	9.7	1.2(3)*	111(12)	40(1)	720	150(2)
2	1+	0	17.	17.0	0.9(3)	37(3)	20(3)	640	345(3)
									II. Diuresis started during treatment,
3a	4+	0	17.	0.7	0.9(2)	96(9)	421(3)	1410	610(2)
3b	3+	0	17.	52.0†	1.0(2)	92(10)	164(2)	2100	1280(1)
4	3+	0	22.	0.7	2.0(1)	80(10)	165(1)	200	165(1)
5a	3+	0	5.	0.4	0.1(3)	40(10)	33(4)	185	87(3)
5b	3+	0	17.	0.8	0.3(5)	95(10)	200(1)	210	123(3)
6	2+	±	34.	39.4†	19.1(1)	149(6)	107(1)	1510	1230(1)
									III. Diuresis prima-
7	4+	0	17.	1.6	0.9(4)	2.5(6)	517(4)	760	450(1)
8	4+	0	5.	0.3	0.1(4)	1.1(11)	99(7)	140	—
9	3+	0	51.	34.0	5.6(2)	34.5(12)	98(7)	1750	770(4)
10	4+	0	9–17	0.3	0.1(2)	1.0(10)	327(5)	480	240(5)
									IV. Diuresis
11	3+	3+	17.	1.8	0.6(5)	2.0(10)	18.0(4)	800	390(1)
12	4+	4+	—	—	—	—	—	900	560(4)
13	4+	4+	—	0.5	—	1.3(9)§	121.0(11)	235	—
14	4+	3+	17.	11.4	0.2(3)	1.0(5)	24.6(5)	1150	360(1)

* Number in parenthesis indicates day of observation.
† Sodium intake above 50 m.eq./day on previous days.

(Table III). The average of these determinations was 5.2 γ, compared with a normal average of 1.5 γ (normal range 0 to 3.4 γ). The restriction of sodium intake may have accounted in part for the abnormally high output of sodium-retaining corticoids in some of the patients, but the data in case 10 suggest that wide variations in dietary sodium did not affect the assay significantly. In the other cases, changes in sodium-retaining activity occurred under treatment with ACTH without any change in the sodium intake.

In two cases, considerable increases in sodium-retaining activity were noted on the second and third days on ACTH, coincident with the decrease in sodium excretion.

In groups 1 and 2, five of the six series of assays showed a distinct fall in sodium-retaining activity during or after administration of ACTH. In the sixth (case 3a), the level of activity had fallen after treatment with cortisone and albumin. No significant change in activity was noted when ACTH was given, but when a second course of ACTH (case 3b) was given six months later because of a recurrence of edema, the sodium-retaining activity responded as in the other cases. In case 5, a more rapid diuresis and a sharper fall in sodium-retaining activity were noted on the second course of ACTH (case 5b).

In contrast to the effects of cortisone, which usually resulted in a diuresis and a fall in sodium-retaining activity after the end of treatment (8), three patients who lost much of their edema during ACTH administration showed a disappearance of the excessive sodium-retaining activity on the ninth to 11th days of ACTH therapy (Table III, cases 1, 3b, 5b). In these patients, there was a rise in sodium-retaining activity on the first or second day after the end of ACTH administration.

TABLE II (*Continued*)

volume treatment		Serum sodium concentration				Creatinine clearance			Case No.
	After B maximum (*cc./day*)	Control (*m.eq./L*)	During treatment		After B maximum (*m.eq./L*)	Control average (*cc./min./1.73 m.²*)	On B maximum (*cc./min./1.73 m.²*)	After B maximum (*cc./min./1.73 m.²*)	
Maximum (*cc./day*)			Minimum (*m.eq./L*)	Maximum (*m.eq./L*)					
during treatment									
1340(12)	1230(2)	140.5	130.0(3)	140.0(10)	140.0(3)	44	58(10)	52(1)	1
1160(9)	1180(2)	142.0	140.0(9)	141.5(2)	141.5(6)	64	80(9)	81(1)	2
completed after end of treatment									
4810(5)	4675(3)	128.0	127.0(4)	146.0(9)‖	146.0(1)	46‡	97(9)	113(1)	3a
4160(10)	4200(2)	143.0	141.0(3)	144.5(6)	143.0(21)	102	123(8)	107(2)	3b
686(10)	1270(1)	138.0	138.0(2)	145.0(9)	143.0(2)	39	78(10)	86(4)	4
275(8)	440(6)	135.0	127.5(5)	138.0(11)	140.0(4)	40	68(12)	82(2)	5a
1350(10)	2150(1)	132.5	127.5(4)	141.5(11)	142.5(2)	52	63(10)	73(4)§	5b
3450(6)	2320(3)	141.0	139.0(6)	142.0(4)	137.0(3)	64‡	87(10)	86(1)	6
rily post-treatment									
1450(9)	5380(4)	135.0	125.5(5)	127.0(3)	137.0(8)	25	32(7)	36(4)	7
500(9)	1230(6)	130.0	120.0(7)	131.5(10)	144.0(13)	29	32(7)	37(5)	8
2400(7)	4320(3)	131.0	117.0(14)	123.0(7)	135.5(9)	45	68(13)	70(1)	9
1415(9)	3800(5)	116.0	114.5(2)	132.0(10)	140.5(4)	60	96(10)	97(11)§	10
ineffectual									
1080(3)	2150(1)	138.0	132.0(6)	133.5(8)	139.0(4)	22‡	25(p)‖	43(p)‖	11
910(3)	2015(6)	138.0	137.0(3)	140.0(8)	139.0(5)	Serum creatinine = 3 mg.%			12
455(9)	1045(11)	138.0	—	141.0(11)	—	Serum creatinine = 0.8 mg.%			13
680(5)	2485(1)	137.5	123.0(11)	134.0(2)	138.0(9)	27‡	40(8)	39(1)	14

‡ Modified by recent administration of cortisone.
§ Intercurrent infection.
‖ (p) = pooled specimen.

In group 3, the time of diuresis and of fall in sodium-retaining activity resembled the events after successful use of cortisone, when maximal diuresis and fall of sodium-retaining activity to low levels was noted on the fourth or a later day after the end of treatment (Table III, cases 7, 10).

Serum potassium concentration was not greatly affected by treatment. In an effort to avoid a sharp rise in serum potassium during treatment, similar to that occasionally observed during treatment of nephrosis with cortisone (8, 10), a reduction in potassium intake by the elimination of foods very high in potassium was made in the present study. Perhaps as a result of the smaller potassium intake, or perhaps because of other differences in the actions of ACTH, the trend of the present group was toward a lower serum potassium during treatment. In five patients, the concentration of potassium fell below 4 m.eq./L in the serum, the lowest level being 3.1 m.eq./L. An increased potassium intake prevented further fall in the concentration of potassium in the serum.[2] The serum potassium level returned promptly to normal when treatment was stopped. The level of potassium in the serum is apparently regulated with difficulty during treatment, and should be carefully followed.

Proteinuria was sometimes increased above control levels during treatment with ACTH. Subsequently, it was greatly reduced in ten of the 15 courses of ACTH in 14 patients (Table I). In

[2] In one patient observed after the completion of this report, the serum potassium fell from 4.0 to 1.9 m.eq./L in two days of rapid diuresis during ACTH administration. Intensive replacement of potassium and withdrawal of ACTH were necessary to control the sharp fall in serum potassium concentration.

four patients, the urinary protein reached a minimum of 100 mg. or less per day. In general, reduction of proteinuria occurred at about the same time as diuresis. Although diuresis might occur without reduction of proteinuria, proteinuria was not observed to fall without diuresis. Thus, in groups 1 and 2, seven of the eight cases showed reduction of proteinuria during treatment, although a minimum was usually reached after the end of treatment. In group 3, only one patient showed a reduction in proteinuria during treatment, while in the other three patients, the reduction of proteinuria, like diuresis, occurred after the end of treatment. Proteinuria was unchanged in group 4.

Concentration of serum proteins and albumin were significantly increased in 11 of the 12 cases in which diuresis was observed (Table I). In four cases, the levels of total protein and albumin approached normal. The average changes in groups 1, 2, and 3 were: total protein increased from 4.0 to 5.3 gm./100 cc. and albumin increased from 1.6 to 2.8 gm./100 cc.

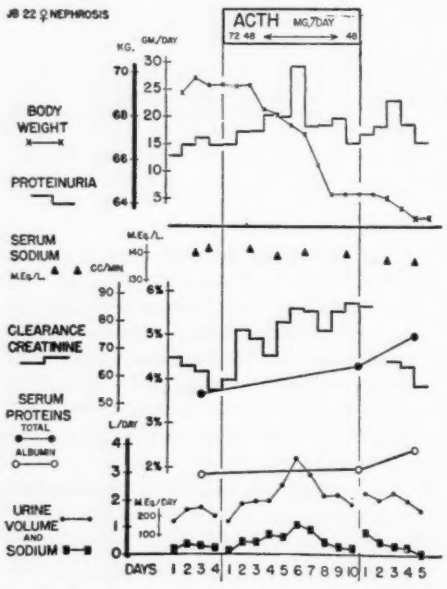

FIG. 3. PARTIAL DIURESIS DURING ADMINISTRATION OF ACTH (CASE 6)

A second peak of sodium excretion and weight loss occurred after ACTH was withheld. Proteinuria was not significantly affected, but serum protein levels were increased.

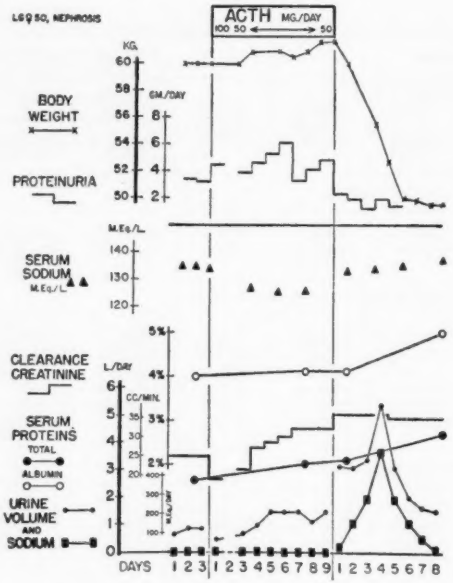

FIG. 4. DIURESIS AFTER END OF ADMINISTRATION OF ACTH (CASE 7)

No clinical evidence of improvement appeared during treatment, although creatinine clearance and serum proteins showed slight increases. After the end of treatment, a brisk diuresis ushered in a remission which has continued for ten months, with normal blood proteins and no abnormality of the urine.

It is of some interest that considerable increases in total protein or albumin concentration may occur while proteinuria continues at a steady or increased rate. A part of the increased concentration may be due to the shrinkage of extracellular fluid volume during diuresis (*e.g.*, case 6), but in cases 11, 12, and 13, the improvement in albumin or protein concentration without diuresis or decrease in proteinuria suggests an increased production or release of proteins into the plasma.

Microscopic examination of the urinary sediment showed a disappearance of abnormal forms after treatment in cases 1, 2, and 4. These patients excreted red blood cells (1 to 4 million/24 hours), epithelial cells containing fat, and a variable number of hyaline and granular casts before treatment. After treatment, these abnormalities were consist-

TABLE III

*Sodium-retaining activity of urinary corticoid fraction**
(expressed in equivalents in micrograms of desoxycorticosterone per 20 minutes of urine)

Case No.	Day of urine collection	Clinical status	Sodium-retaining activity (γ)	Urine volume (*cc./day*)	Urine sodium (*m.eq./day*)	Serum sodium (*m.eq./L*)
1	Control	Heavy edema	4.4	853	5.0	140
	On ACTH day 3	Edema worse	7.2	184	1.2	130
	On ACTH day 11	Lost 2.6 kg.	0.7	1085	97.0	140
	After ACTH day 1	No edema	0.8	975	40	140
3a	Control	Heavy edema	4.3	405	0.4	133
	After cortisone and albumin	Little change	2.4	1230	0.6	130
	On ACTH day 7	Diuresis water	2.5	2405	11.3	141
	After ACTH day 3	Peak of diuresis	2.7	4675	421.	142
3b	Control	Recurrence of edema	3.4	2030	50.	143
	On ACTH day 2	Unchanged	5.2	2060	1.0	141
	On ACTH day 9	Lost 1.4 kg.	0.5	3150	49.	144
	After ACTH day 2	End of diuresis	3.6	4200	164.	142
4	Control	Heavy edema	3.8	115	0.5	138
	After ACTH day 2	Lost 2.4 kg.	2.6	1025	113.	143
5a	Control	Edema 3+	5.2	144	1.9	138
	After diuretic	Unchanged	7.3	170	0.3	135
	On ACTH day 5	Edema worse	8.9	152	0.1	128
	On ACTH day 10	Lost 0.3 kg.	4.1	263	40.2	137
	After ACTH day 1	Lost 0.6 kg. total	5.7	240	2.2	138
	After ACTH day 4	No visible edema	3.1	282	33.3	140
5b	Control	Edema 3+	5.6	257	1.3	133
	On ACTH day 5	Edema worse	5.0	190	0.3	129
	On ACTH day 10	Lost 1.4 kg.	0†	1350	95.	140
	After ACTH day 1	Peak of diuresis	3.3	2150	200.	142
7	Control	Heavy edema	5.5	823	2.0	134
	After ACTH day 4	Peak of diuresis	0†	5380	517.	134
10	Regular diet Paracentesis	Heavy edema	9.6	208	0.2	135
	Na intake 9m.eq.	Edema same	9.5	850	0.5	116
	On ACTH day 2 Na added to diet	Edema same	7.7	680	0.1	115
	On ACTH day 8	Edema same	6.6	1415	0.5	125
	After ACTH day 4	Rapid diuresis	1.1	2630	316.	140

* See Addendum, p. 1541
† Caused increased sodium excretion in rats.

ently absent. In three other cases, no abnormality was noted before or after treatment. Eight other patients showed no change in number or type of abnormal cells or casts observed before treatment.

Serum cholesterol was frequently decreased during treatment (Table I) and returned gradually toward normal after treatment when improvement continued.

Hematocrit was insignificantly affected during or after treatment. An occasional striking improvement is masked in the averages by declines in other cases, usually small children. The repeated drawing of blood for chemical analysis may have affected these results.

Erythrocyte sedimentation rate was reduced after treatment only in cases 3 and 6. In the other patients, the rapid sedimentation was unaffected.

White blood cells were increased in number during administration of ACTH from a control average of 8,600/c.mm. to an average peak of 21,600/c.mm. during treatment. Counts returned to normal within a few days after treatment.

Eosinophilic leukocytes were found in the blood in numbers between 100 and 300/c.mm. in eight

TABLE IV

Comparison of clearances of creatinine and inulin during and after diuresis in case 3b

Day of test	Clinical status	Clearance		
		Inulin (a.m.)*	Creatinine (a.m.)	Creatinine (24-hour)
		(*cc./min./1.73 sq.m.*)		
Control	Edema 3+	112	111	102
ACTH day 7	Beginning diuresis	133	121	115
ACTH day 10	Increasing diuresis	132	119	122
3 days after last dose of ACTH	End of diuresis	121	116	96

* (a.m.) indicates the average clearance during three periods of 30 minutes each on the morning of the day noted.

of the patients before treatment. In five of the patients, there were 325 to 2850 eosinophiles/c.mm. Two patients had counts below 100/c.mm. During treatment eosinophiles fell to between 0 and 11 cells/c.mm. in 11 patients. In terms of percent of counts before treatment, eosinophiles were reduced to less than 10% of the control level in 13 of 15 courses of treatment. Cases 4 and 12 showed reductions to 30 and 66%, respectively, of the control levels.

It was of some interest that the number of eosinophiles tended to fall sharply and then to rise slowly in some patients who showed a diuresis during the later days of therapy. Some observations on urinary 17-ketosteroid excretion by two of these patients, as well as a few reported observations by other workers (6, 11), do not support the suggestion that there might be a decline of adrenal secretion at this time.

Blood pressure was increased in ten patients during treatment, essentially unchanged in three, and slightly decreased in one patient. The average blood pressure in these patients was 120/80 before treatment and 132/85 at the end of administration of ACTH. Either systolic or diastolic pressure might be affected. Maxima observed were 170 systolic and 110 diastolic. In ten patients, blood pressure during the five days after the end of treatment fell below the pre-treatment level by an average of 15 mm. Hg. In the rest of the group, blood pressure was not affected or it returned gradually to control levels, remaining elevated above the control level for as long as a week in one patient.

Duration of effects. Three of the ten patients (cases 2, 4, 7) who lost all edema during or after the first course of administration of ACTH are in remission three to ten months later, with diminished proteinuria and freedom from edema.

Seven patients have had a recurrence of edema, beginning from one to four months after treatment. In four of these patients (cases 1, 5, 8 and 10), a severe respiratory infection was followed by increased proteinuria and appearance of edema of slight to moderate degree. Three other patients have had a gradual return of edema, attributed by the patient to posture (sitting at a desk in school), to exposure to sun, or to no evident reason. In most of these cases, the edema has been quite moderate in extent, sometimes being present only during or after a respiratory infection. Appetite and general condition have remained excellent.

A second course of ACTH has been given to three of these patients (cases 3, 5, 9) because of persistent edema and gradual deterioration of appetite, activity, and psychological outlook. The response to treatment was similar to that after the first course. A greater reduction in proteinuria was noted on the second trial of ACTH in case 3b, while edema was more promptly and completely eliminated in case 5b. Insufficient data are available for an exact comparison of the second course of ACTH in case 9, but the clinical response was similar in all respects.

The three patients who received a second course of ACTH are free of edema. Case 9 has been treated too recently to allow any evaluation. In cases 3 and 5, reduced proteinuria and freedom from edema have continued for two and five months, respectively, after treatment.

DISCUSSION

The mechanism by which improvement occurs during or after treatment of nephrosis with ACTH is complex, but certain results of treatment are clear.

The initial effect of ACTH is often an aggravation of the clinical picture of nephrosis. Edema and proteinuria are usually increased, urine volume falls, and there may be a temporary reduc-

tion of "creatinine" clearance. Abnormalities of the urinary sediment may be even more evident in the highly concentrated urine. These changes occur at a time when the symptoms of rheumatic or "collagen" disease would be clearing. In case 9, for example, the general signs of lupus erythematosus disseminatus were promptly improved by ACTH, while the evidences of the renal lesion were unchanged or aggravated.

After some days of treatment, signs of improvement appear. In about half of the cases, profuse diuresis and progressive loss of edema are seen during treatment. Several factors which may affect diuresis are modified at this time. These include the clearances of "creatinine" and of inulin, the serum sodium concentration, and the sodium-retaining activity of the corticoid fraction of urine.

There is a considerable increase in the clearance of "creatinine" in most of these patients, as has been previously observed (7). This improvement usually reaches a peak near the end of treatment, and subsequently declines. A variable degree of the gain in "creatinine" clearance is maintained.

The changes are larger in magnitude and more persistent than those observed in patients without renal disease treated with ACTH (12, 13). There is no clear correlation between any given level of clearance and clinical improvement.

A subnormal concentration of sodium in the serum of an edematous patient, reflecting an excess of water over sodium in extracellular fluid, occurs commonly in patients with nephrosis. During the first days of treatment with ACTH, this situation may be aggravated. The adrenal 11-oxysteroids, excreted in increasing quantities under the influence of ACTH (6, 11), have an appreciable diuretic action under similar conditions (14). This action could account for the diuresis of water and the subsequent rise in the serum sodium concentration, which usually precede an effective diuresis.

Measurement of the sodium-retaining activity of the corticoid fractions of the urines of this group of patients with nephrosis shows greater than normal activity before treatment. This abnormally high sodium-retaining activity may be increased initially when ACTH is given, and then falls when diuresis occurs. Similar observations in patients treated with cortisone show a reduced sodium-retaining activity during the diuresis which often follows after cortisone administration. The hypothesis that cortisone suppresses the output of an active sodium-retaining hormone has been offered as a possible explanation. The fall in urinary sodium-retaining activity which accompanies the diuresis during the second week of ACTH administration can not be satisfactorily explained in this way, since studies of other adrenal secretions show a continued or increasing level of adrenal activity at this time. Further studies are necessary to evaluate hypotheses concerning changes in metabolism (15), competitive interactions, and variations in responsiveness of the renal tubules (2).

When reduction in proteinuria and improvement in urinary sediment occur, these changes appear at approximately the same time as the diuresis. It seems probable that all of these events are mediated by closely related factors, as yet ill-defined.

The improvement in the concentrations of albumin and of total proteins in the serum is generally greatest if both a diuresis and a reduction in proteinuria occur. Reduction of proteinuria does not seem to be a necessary factor for improvement in the concentration of serum proteins, however. In several cases, a virtual cessation of proteinuria was followed by a very disappointing improvement in serum protein levels. As in other forms of treatment, proteinuria and hypoproteinemia are no obstacle to diuresis, while maximum improvement in serum proteins occurs after the end of the diuresis (9, 16).

Moderate increases in plasma volume have been observed in children with nephrosis during treatment with ACTH (7). Significant changes were associated with proportional reduction in the hematocrit. The hematocrit was usually unchanged or moderately increased in adult patients in the present study. It seems unlikely that any large increase in plasma volume occurred, unless this was masked by a simultaneous and proportional increase in red blood cell volume. Further studies of this possibility are indicated.

The effect of these changes on the ultimate course of the disease remains to be evaluated by follow-up for a longer period of time. In the meanwhile, the clinical manifestations of the disease have been greatly improved. Most of these

patients are leading an essentially normal life except for continued restriction of sodium intake.

A comparison of the effects of ACTH and cortisone shows many points of similarity. A few differences appear to be consistent. The administration of ACTH is followed by diuresis in a higher proportion of cases than in a similar group treated with cortisone. Diuresis may appear during treatment with ACTH, while it rarely appears until after the end of cortisone administration. Reduction of proteinuria below the control level occurs commonly during ACTH treatment, while such improvement is noted less frequently after the withdrawal of cortisone. The fall in serum cholesterol is much more striking after administration of ACTH. Increases in blood pressure are more impressive during ACTH than during cortisone treatment. In the dosage used, ACTH appears to be more effective than cortisone in inducing an immediate improvement in a larger proportion of cases, but the difference in frequency of response is not so great as might be inferred from scattered reports of a few cases, some of whom were inadequately treated with cortisone. In larger groups of unselected patients, the frequency of diuresis is only moderately less after cortisone than after treatment with ACTH (8, 10). Further study of cortisone, ACTH, and related compounds and of different plans of treatment is indicated.

SUMMARY

When ACTH was administered for nine to 14 days to 14 patients with nephrosis, the following results were noted:

(*1*) Almost all patients retained water and gained weight. There was reduction of urinary sodium excretion and increased proteinuria. In this group of patients on a small sodium intake, the concentration of sodium in the serum fell during this phase, which lasted from one to 14 days.

(*2*) In six cases, increasing diuresis appeared on the second to sixth day of ACTH treatment, and was followed by a rise in the serum sodium concentration and gradually increasing excretion of sodium in the urine. This diuresis resulted in the elimination of most of the edema. A second increase in diuresis and loss of edema occurred after treatment was stopped.

(*3*) In four cases, no effective diuresis appeared during nine to 14 days of treatment, but there was complete elimination of edema by a rapid diuresis beginning immediately after the end of administration of ACTH.

(*4*) In four cases, no significant clinical improvement was observed.

(*5*) Proteinuria was reduced in eight of the ten patients who lost their edema. In four cases, the excretion of protein in the urine fell to normal levels.

(*6*) All abnormalities of the urinary sediment disappeared in three cases.

(*7*) There were increases in the concentrations of protein and albumin in the serum, apparently related in part to diuresis and to reduction in proteinuria, but not always explicable on these bases.

(*8*) The abnormally high concentration of cholesterol in the serum was usually decreased.

(*9*) There was increased clearance of endogenous "creatinine" during treatment. After treatment, "creatinine" clearance usually decreased, but some improvement above control levels was maintained.

(*10*) The abnormally high sodium-retaining activity of the urinary corticoids decreased at the time of diuresis during or after treatment.

(*11*) The blood pressure was variably increased during treatment, but usually fell below control levels after the end of treatment.

(*12*) The concentration of potassium in the serum varied either above or below normal in different patients. These variations were difficult to predict, but, once determined, could probably be minimized by appropriate changes in potassium intake.

(*13*) The clinical improvement induced by ACTH was generally fully maintained for a few months, after which a recurrence of the nephrotic syndrome might follow a minor infection or might appear without obvious cause. Remissions have persisted for many months.

(*14*) A second course of treatment with ACTH was effective in the treatment of recurrent edema after the first course of cortisone or ACTH.

CONCLUSIONS

1. The administration of ACTH is followed by elimination of edema and by improvement in the other abnormalities of nephrosis in a large proportion of the cases treated.

2. Certain physiological changes are observed regularly to precede or accompany the elimination of edema. The most important are increasing renal clearance of "creatinine," increasing urine flow, increasing serum sodium concentration, and decreasing sodium-retaining activity of the urinary corticoids. No one of these physiological measurements appears to be the sole regulator of diuresis. Elimination of edema seems to be the result of a favorable combination of the important factors, which may occur during or immediately after the administration of ACTH.

4. The parallel changes in edema and proteinuria, both increasing in the first days of ACTH treatment, and both usually decreasing simultaneously whether during or after treatment, suggest that these abnormalities are regulated by closely related mechanisms. The concentration of the plasma proteins seems to be affected secondarily.

5. The modification of the most important physiological manifestations of the nephrotic syndrome by the adrenal cortical steroids is again demonstrated. The exact role of these steroids in aggravating or improving the renal disturbance remains uncertain.

ACKNOWLEDGMENT

The authors wish to express their gratitude to the visiting and resident staffs of Stanford University Hospitals for referral of patients and for assistance in their care; to the nurses of the medical and pediatric wards; to the dieticians for the special diets; to Armour Laboratories for ACTH; to Dr. Edward Henderson of Schering Corp. for desoxycorticosterone; and to Dr. F. S. Greenspan for assistance with determinations of 17-ketosteroids.

ADDENDUM

Since this report was prepared, some observations show that the solvent in which the standard DCA and the unknown are dissolved has an appreciable sodium-retaining effect in the method of bioassay used, although it was thought to be inert on the basis of previous controls with a less sensitive method. This systematic error does not affect values about 5 γ, but adds an increasing blank to less active materials. No change in interpretation appears necessary, since correction of the error would result in more impressive changes of the type described.

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HEMAGGLUTININS AND HEMOLYSINS FOR ERYTHROCYTES SENSITIZED WITH TUBERCULIN IN PULMONARY TUBERCULOSIS [1]

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The recent discovery by Middlebrook (1, 2) that sera from tuberculous animals and human beings are capable of agglutinating and lysing erythrocytes previously treated with extracts of tubercle bacilli has aroused considerable interest. In a brief period of time several clinical evaluations of the hemagglutination test have appeared in print (3, 4). As yet only one preliminary report has appeared concerning its hemolytic modification (4). It is the purpose of the present paper to report our clinical experience with both tests during the past year. More than 1000 tests have been carried out in that period with sera obtained from well over 100 patients having active tuberculosis and a similar number of adults not having active tuberculosis.

MATERIAL

The tuberculous patients selected for this study were hospitalized either on the Tuberculosis Service of the Minneapolis Veterans Administration Hospital or at the Minneapolis General Hospital. Pulmonary tuberculosis was predominant, and in most cases the disease was extensive. The diagnosis was proved in all cases roentgenologically and by isolation of *M. tuberculosis* from the sputum or gastric washings. A total of 151 patients having active pulmonary tuberculosis was studied; several of the patients had extra-pulmonary dissemination of their tuberculosis, but in most the pulmonary infection was predominant. The control group included 128 subjects having a negative chest roentgenogram. The group included college students, physicians, medical technicians, and a few patients having non-tuberculous diseases. All were tested for tuberculin sensitivity,[2] and 61 were found positive while 67 were negative. Thirty-four of the latter group were entering freshmen students at the University of Minnesota. No individual was included who had received a Mantoux test during the previous three months in order to avoid antibody titers resulting from such antigens (5).

METHODS

Hemagglutinins. The method of Middlebrook and Dubos (1) was employed. Heterophile antibodies were absorbed from the heat-inactivated sera by the method of Middlebrook (2). Sheep cells were collected in modified Alsever's solution, and streptomycin (1000 units per ml.) was added as a preservative. Old tuberculin, 4× concentrated,[3] was used to sensitize the sheep cells as recommended by Scott and Smith (6). Sensitized sheep cells were added to serial twofold dilutions (1:4 through 1:256) of the absorbed sera. Appropriate controls were included. After incubation for two hours at 37° C., and overnight at room temperature, the degree of hemagglutination was recorded after resuspension.

Hemolysins. The method of Middlebrook (2) was used and differed little from that given above. Fresh guinea pig serum diluted 1:3 and absorbed with sheep cells was used as the source of complement. After addition of tuberculin-sensitized sheep erythrocytes and complement to the serially diluted patient's serum, incubation was carried out at 37° C. for one hour. The degree of hemolysis was then recorded.

Coombs Hemolysins. A sensitive hemolytic modification of the indirect Coombs test (7) was devised (4). Human (group O, Rh negative) erythrocytes were used in place of sheep cells in order that difficulties with heterophile antibodies might be avoided. Serial twofold dilutions of heat-inactivated immune serum were prepared (1:2 through 1:128). To 0.1 ml. of each was added 0.1 ml. of a 2 per cent suspension of tuberculin-sensitized erythrocytes. After incubation for one hour at 37° C. the erythrocytes were thrown down by high speed centrifugation and carefully washed three times with ten volumes of buffered 0.8 per cent saline solution (pH 7.0). After resuspension to their original volume in saline, the erythrocytes were added to an equal volume

[1] Sponsored by the Veterans Administration and published with the approval of the Chief Medical Director. The statements and conclusions published by the authors are the result of their own study and do not necessarily reflect the opinion or policy of the Veterans Administration.

[2] 0.1 ml. of 1:1000 dilution of old tuberculin intradermally.

[3] Lot no. 2725-2 obtained from Lederle Laboratories, Pearl River, N. Y., through the courtesy of Dr. H. D. Piersma.

of Coombs anti-human globulin rabbit serum [4] and incubated one hour at 37° C. Fresh guinea pig complement [5] (0.05 ml. 1:3 dilution) was then added and a final incubation carried out for one hour at 37° C. Control tubes were included in which Coombs serum was replaced by heat-inactivated, absorbed normal rabbit serum as well as a control tube in which unsensitized erythrocytes replaced the tuberculin-treated human cells. Hemolysis was recorded immediately after the final incubation.

"Blocking" antibodies. Two methods were used to detect the presence of "blocking" antibodies. In method No. 1 an equal quantity of the suspected blocking serum was added to serial twofold dilutions of an immune serum having a high antibody titer. Hemagglutinin and hemolysin tests were then performed in the usual manner. In method No. 2 the reverse procedure was carried out. That is, the amount of the immune serum was kept constant and the quantity of blocking serum varied.

RESULTS

The hemagglutinin antibody titers of 151 patients having active pulmonary tuberculosis are shown in Table I. The median titer was 1:8; only 9.3 per cent had a titer greater than 1:32. In 128 subjects having a negative chest roentgenogram the median hemagglutinin titer was less than 1:4. There was no difference in the median titer of the control subjects having a positive Mantoux test as compared with those having a negative Mantoux test. There was no clinical evidence that any of these control subjects had active tuberculosis, yet several had a hemagglutinin titer of 1:8 or higher.

The hemolysin antibody titers of the majority of these subjects were determined and are also presented in Table I. The median hemolysin titer of 115 patients having active pulmonary tuberculosis was 1:32 and in 112 control subjects having a negative chest roentgenogram was less than 1:4. The presence of tuberculin hypersensitivity appeared to have no influence upon the median hemolysin titer of the control subjects. A considerable number of the control subjects had a hemolysin titer of 1:32 or greater, particularly among those whose Mantoux test was positive.

We have published elsewhere preliminary data concerning the simultaneous determination of hemagglutinin and hemolysin titers (4). In 68.7 per cent of 103 patients having active pulmonary tuberculosis and 105 healthy control subjects, the two tests revealed antibody titers which did not differ by more than one dilution. In 29.3 per cent the hemolysin titer exceeded the other by more than one dilution, and in only 1.9 per cent was the hemagglutinin titer significantly greater than the hemolysin titer. Serial observations indicated that the latter phenomenon was usually only transient, and complement titration proved that it was not due to the presence of an anti-complementary substance.

All patients included in this study had active pulmonary tuberculosis. The extent of their pulmonary disease did not appear to influence their antibody titer. In a group of 127 patients tested for hemagglutinins, there were 13 with minimal, 53 with moderately advanced, and 61 with far advanced pulmonary tuberculosis. The median hemagglutinin titer was 1:4 in the group having far advanced pulmonary tuberculosis, and 1:8 in each of the other two groups. In a study of 103 of the same patients the hemolysin test yielded a median titer of 1:32 in each of the three groups.

As can be seen in Table I, patients having active pulmonary tuberculosis frequently possessed

[4] Prepared by Ortho Pharmaceutical Corp., Raritan, N. J.

[5] Absorbed with human (group O, Rh negative) erythrocytes (2).

TABLE I

Anti-tuberculin hemagglutinins and hemolysins

Antibody titer	Active pulmonary tuberculosis		Control subjects			
			+Mantoux		−Mantoux	
	No.	%	No.	%	No.	%
Hemagglutinins						
>128	4	2.6	0	0	0	0
128	1	0.6	0	0	0	0
64	9	5.9	0	0	1	1.5
32	21	13.9	3	4.9	0	0
16	28	18.5	4	6.5	2	3.0
8	22	14.6*	6	9.8	4	6.0
4	31	20.5	9	14.7	20	29.8
<4	35	23.2	39	63.9*	40	59.7*
Total	151		61		67	
Hemolysins						
>128	18	15.6	1	2.0	1	1.6
128	8	7.0	1	2.0	0	0
64	16	13.9	1	2.0	2	3.2
32	20	17.4*	7	14.0	3	4.8
16	17	14.7	5	10.0	7	11.3
8	15	13.0	7	14.0	7	11.3
4	12	10.4	3	6.0	8	12.9
<4	9	7.8	25	50.0*	34	54.8*
Total	115		50		62	

* Median.

TABLE II

Anti-tuberculin Coombs hemolysins in patients having active pulmonary tuberculosis but negative Middlebrook tests (1, 2)

Subjects	Serum dilutions (reciprocal)							
	C*	2	4	8	16	32	64	128
	With Coombs anti-human globulin rabbit serum							
Normal Patients	0**	0	0	0	0	0	0	0
O. Lo.	0	4+	4+	4+	4+	4+	4+	3+
Wo.	0	4+	4+	4+	4+	4+	3+	2+
O'C.	0	4+	4+	4+	4+	4+	3+	2+
Wa.	0	4+	4+	4+	4+	3+	2+	2+
Re.	0	4+	4+	4+	3+	1+	1+	0
Bo.	0	1+	1+	1+	1+	1+	1+	0
	With normal rabbit serum (control)							
Normal Patients	0	0	0	0	0	0	0	0
O. Lo.	0	1+	1+	1+	1+	0	0	0
Wo.	0	2+	2+	0	0	0	0	0
O'C.	0	0	0	0	0	0	0	0
Wa.	0	0	0	0	0	0	0	0
Re.	0	0	0	0	0	0	0	0
Bo.	0	0	0	0	0	0	0	0

* Unsensitized sheep cell control.
** 0 to 4+ = hemolysis after one hour at 37° C.

little or no hemagglutinin in their serum and somewhat less often lacked hemolysin as well. An exhaustive search for "blocking" antibodies in such sera largely failed. In no instance were we able to disclose blocking of hemagglutinins, and the serum of only one patient inhibited the hemolytic activity of a potent immune serum (4). The addition of bovine albumin or normal human serum in a final concentration of 15 per cent, moreover, did not increase the hemagglutinin or hemolysin activity of low titer sera obtained from patients having active pulmonary tuberculosis.

The addition of Coombs anti-human globulin rabbit serum to sera containing little or no hemagglutinin activity did not substantially increase the hemagglutinin titer of the latter. Coombs anti-human globulin rabbit serum, however, did disclose hemolysins in high titer in almost all sera obtained from patients having active pulmonary tuberculosis even though the Middlebrook test was negative (Table II). Control tests using normal rabbit serum in place of Coombs serum were essentially negative and indicated that the hemolysis was produced by specific human anti-tuberculin hemolytic antibody.

DISCUSSION

The technical aspects of the Middlebrook tests have been discussed elsewhere and need no further emphasis (8). It should be noted, however, that the hemolytic test is quicker and much easier to read than the hemagglutination test. The data presented in Table I, moreover, indicate that the hemolytic test is a more sensitive and no less specific test for anti-tuberculin antibody in human sera.

As demonstrated in Table III, in our hands neither the hemagglutination nor the hemolysis test has shown as great sensitivity and specificity for active tuberculosis as that reported by Rothbard, Dooneief, and Hite (3). It can be readily seen from the table that our results are essentially in agreement with the data given by most other investigators who have used the hemagglutination test. The hemagglutination test is more likely to be positive in a titer of 1 : 8 or greater in the presence of active pulmonary tuberculosis than in subjects whose chest roentgenogram reveals no evidence of tuberculosis. Yet in an individual patient, the test cannot be relied upon by itself as an index of the extent or activity of tuberculosis. In our studies the test was "negative" in 43.9 per cent of 151 patients whose pulmonary tuberculosis was active. Hemagglutinins and hemolysins were found more often in a significant titer [6] in the serum of healthy adults whose tuberculin skin test was positive than in those who were not hypersensitive. Even a minimal sub-clinical pulmonary or extrapulmonary tuberculous lesion may suffice to provoke anti-tuberculin antibodies. In many the presence of these antibodies remains unexplained.

Middlebrook (2) came to the conclusion that human anti-tuberculin hemagglutinin and hemolysin were not identical since their respective titers varied at random in a small group of immune sera. Our own data gathered from a large number of human sera revealed a close correlation between the two; the hemolysin titer frequently exceeded the hemagglutinin titer, but rarely was the hemag-

[6] A titer of 1 : 8 was taken to be significant for hemagglutinins and 1 : 32 for hemolysins.

TABLE III

Summary of results of Middlebrook tests (1, 2) for anti-tuberculin antibodies in human tuberculosis

Author and year	Active pulmonary tuberculosis		Control subjects: "normal" adults			
			+Mantoux		−Mantoux	
	No. of patients	*1:8 or greater* (%)	*No. of subjects*	*1:8 or greater* (%)	*No. of subjects*	*1:8 or greater* (%)
Hemagglutinins						
Rothbard, Dooneief, and Hite (3) 1950	168	92.3	33*	0	110**	0
Smith and Scott (5) 1950	24	70.8				
Gernez-Rieux and Taquet (9, 10) 1949, 1950	504	79.8	43	0	244**	6.9
Sohier (11) 1951	27	74.1	74	16.2	47	14.9
Fleming, Runyon, and Cummings (12) 1951	109	66.1	18	44.4	35	20.0
McDearman (13) 1951	56	71.4			41**	4.8
Kirby, Burnell, and O'Leary (14) 1951	42	69.0				
Total	930	79.0	168	11.9	477	7.1
Hall and Manion 1951	151	56.1	61	21.2	67	10.5
		1:32 or greater (%)		*1:32 or greater* (%)		*1:32 or greater* (%)
Hemolysins						
Hall and Manion 1951	115	53.9	50	20.0	62	9.6

* "Cured tbc." ** "Normal"

glutinin titer the greater by more than one dilution.

Our data do not permit an evaluation of the effect of tuberculous activity upon antibody titer since the disease was active in the great majority of our patients. The median antibody titer was not significantly influenced, however, by the extent of the pulmonary tuberculosis. In patients with far advanced pulmonary tuberculosis the median hemagglutinin titer was slightly less than in patients whose pulmonary disease was less extensive. Sufficient sera from patients with predominantly extrapulmonary tuberculosis have not been tested to permit reliable conclusions.

Hemagglutinins and hemolysins were frequently low in titer and occasionally absent in some patients with active pulmonary tuberculosis. This was not due to the presence of "blocking" antibodies. Nor did such sera contain demonstrable anti-complementary substances. The addition of protein did not disclose greater antibody activity. The addition of Coombs anti-human globulin rabbit serum, however, did disclose anti-tuberculin hemolysin in almost all such sera in high titer (Table II). These sera apparently contained "incomplete" antibody which was capable of uniting with tuberculin adsorbed upon erythrocytes, yet was incapable of agglutinating the cells. Sera of this type also were not able to bind complement in the presence of tuberculin-sensitized erythrocytes; this probably accounts for their low hemolytic activity. Such sera did cause slow hemolysis without complement being bound (8). The slight hemolytic activity of these sera for tuberculin-sensitized erythrocytes was due to an "incomplete" anti-tuberculin antibody. The latter was proved by means of a hemolytic modification of the indirect Coombs test (Table II). The lack of "blocking" activity by these sera was an unexpected finding. Despite its union with tuberculin, the antibody in these sera apparently did not block the receptors for "complete" anti-tuberculin antibody. We have likewise found with anti-Brucella sera that Coombs serum is a more sensitive indicator of "incomplete" antibody than are "blocking" tests (unpublished studies).

The reason for the occurrence of "incomplete" antibodies in human tuberculosis has not been disclosed. They have never been found in normal adults and have been associated particularly with chronic, far advanced, pulmonary tuberculosis.

A similar but somewhat different phenomenon has been produced by hyper-immunization of rabbits with tuberculin (4, 8). Anti-tuberculin antibodies appeared promptly after weekly intravenous injection of large doses of tuberculin. After several weeks titers of anti-tuberculin antibodies declined gradually. Ultimately even Coombs hemolysins disappeared and did not reappear after further tuberculin immunization. The rabbits, however, remained capable of responding to other unrelated antigens (8). The disappearance of anti-tuberculin antibodies was not the result of replacement by "incomplete" antibody. It appeared to be due to an immunologic "paralysis" similar to that reported by Felton (15) in mice immunized with large amounts of pneumococcus polysaccharide. Since human tuberculosis is usually a very chronic infection, it is possible that excess antigen liberated over a long period of time may contribute in a similar manner to the paucity of antibodies in some patients.

SUMMARY AND CONCLUSIONS

1. Hemagglutinins for tuberculin-sensitized sheep cells were found in a titer of 1:8 or greater in the serum of 56.1 per cent of 151 patients having active pulmonary tuberculosis, and in 21.2 per cent of 61 tuberculin-positive as well as 10.5 per cent of 67 tuberculin-negative healthy adults.

2. Anti-tuberculin hemolysins were found in a titer of 1:32 or greater in the serum of 53.9 per cent of 115 of the same tuberculous patients, in 20 per cent of 50 tuberculin-positive controls, and in 9.6 per cent of 62 tuberculin-negative controls.

3. Studies of 208 human sera revealed a close correlation between anti-tuberculin hemagglutinin and hemolysin titers. The hemolytic test was the more sensitive indicator of anti-tuberculin antibody.

4. Anti-tuberculin hemolysins were disclosed in the serum of nearly all patients having active pulmonary tuberculosis by a modification of the indirect Coombs test even though the hemagglutination and hemolysis tests of Middlebrook were negative.

5. The Coombs hemolysin appeared to be an "incomplete" antibody incapable of agglutinating tuberculin-sensitized erythrocytes or of fixing complement yet able to unite with the tuberculin.

6. With the antigens available none of these tests can be regarded as sufficiently reliable for widespread use in detecting the presence of active tuberculosis unless rigidly controlled by other accepted diagnostic criteria. They cannot supplant allergic, bacteriologic, and radiologic methods in the diagnosis.

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AUTHOR INDEX

A

Abel, Harold A. See MENDLOWITZ, TOUROFF, and ABEL 94

Abernathy, Robert. The effect of cortisone on experimental brucellosis 626

Adams, C. H. See PATTERSON, ADAMS, and STEVENSON 665

Adams, John E. See BENTINCK, GORDAN, ADAMS, ARNSTEIN, and LEAKE 200

Addis, T., Barrett, E., Poo, L. J., Ureen, H. J., and **Lippman, R. W.** The relation between protein consumption and diurnal variations of the endogenous creatinine clearance in normal individuals 206

Adlersberg, David, Drachman, Stanley R., Schaefer, Louis E., and **Dritch, Rhoda.** Interrelations of serum lipids in patients treated with cortisone and pituitary adrenocorticotropic hormone (ACTH) 626

Ahrens, Edward H., Jr., and **Craig, Lyman C.** A quantitative approach to separation of lipid mixtures 626

—. See KUNKEL, AHRENS, EISENMENGER, BONGIOVANNI, and SLATER 654

Aikawa, Jerry K., and **Harrell, George T.** The immunophysiology of serum sickness. Alterations in the blood volume and thiocyanate space in relation to the development of humoral antibodies in the rabbit 360

—, **Harrell, George T.,** and **Miller, Treva B.** The immunophysiology of trichinosis. Alterations in the blood volume and the thiocyanate space in relation to the development of humoral antibodies in the rabbit 575

Alanis, Jesus. See CRUMPTON, FRIEDLAND, KUO, SCHNABEL, FOLTZ, PAGE, ALANIS, and HAFKENSCHIEL 634

Albaum, Harry G., Cayle, Theodore, and **Shapiro, Arthur.** Blood levels of adenine nucleotides 525

Albert, Roy E. See EICHNA, FARBER, BERGER, EARLE, RADER, PELLEGRINO, ALBERT, ALEXANDER, TAUBE, and YOUNGWIRTH 1250

Albright, Fuller. See BARTTER, ALBRIGHT, FORBES LEAF, DEMPSEY, and CARROLL 237

Aldrich, Stephen L. See GILBERT, ALDRICH, and ANDERSON — 640

Alexander, Benjamin, Goldstein, Robert, and **Landwehr, Greta.** The labile factor of prothrombin conversion: its consumption under normal and pathological conditions affecting blood coagulation 252

—, **Goldstein, R., Landwehr, G.,** and **Cook, C. D.** Congenital spca deficiency: a hitherto unrecognized coagulation defect with hemorrhage rectified by serum and serum fractions 596

—, **Goldstein, Robert,** and **Landwehr, Greta.** Pseudohypoprothrombinemia and parahemophilia: deficiency of different components essential for rapid prothrombin conversion 627

Alexander, J. Deaver. See EARLE, FARBER, ALEXANDER, and PELLEGRINO 421

—. See FARBER, ALEXANDER, and EICHNA 638

—. See EICHNA, FARBER, BERGER, EARLE, RADER, PELLEGRINO, ALBERT, ALEXANDER, TAUBE, and YOUNGWIRTH 1250

Altemeier, W. A., Coith, C. L., Culbertson, W. R., Logan, M. A., and **Tyteli, A. A.** Toxoid immunization in *Clostridium novyii* gas gangrene 627

Anderson, Lynn. See GILBERT, ALDRICH, and ANDERSON 640

Andres, Reubin. See ZIERLER, ANDRES, and LILIENTHAL 684

Appleton, Janet E. See CLARK, MANNING, and APPLETON 633

Armistead, Eleanor B. See SCHOENBACH, WEISSMAN, and ARMISTEAD 762

Arnstein, L. H. See BENTINCK, GORDAN, ADAMS, ARNSTEIN, and LEAKE 200

Arst, D. B. See LAHEY, ARST, SILVER, KLEEMAN, and KUNKEL 654

Ashenbrucker, Helen. See CARTWRIGHT, HAMILTON, GUBLER, FELLOWS, ASHENBRUCKER, and WINTROBE 161

Assali, N. S., Kaplan, S. A., Fomon, S. J., Douglass, R. A., and **Tada, Y.** The effect of high spinal anesthesia on the renal hemodynamics and the excretion of electrolytes during osmotic diuresis in the hydropenic normal pregnant woman 916

Astwood, E. B. See PAYNE, ROSENBERG, RABEN, CLEROUX, and ASTWOOD 665

Atwell, R. J. See HICKAM, PRYOR, PAGE, and ATWELL 503

Ayala, Washington, Moore, Lucile V., and **Hess, Eugene L.** The purple color reaction given by diphenylamine reagent. I. With normal and rheumatic fever sera 781

B

Bader, Mortimer E. See LEVITT and BADER 655

Bailey, Mary Lynch. See SALK, BAILEY, and LAURENT 669

Barbero, Guilio. See BARNETT, FORMAN, MCNAMARA, MCCRORY, RAPOPORT, MICHIE, and BARBERO 227

Barker, Harold G. See CLARK, CROSLEY, and BARKER 633

—. See CLARK and BARKER 745

Barker, S. B., Humphrey, M. J., and **Soley, M. H.** The clinical determination of protein-bound iodine 55

Barnes, Benjamin A. See LEVER, GURD, UROMA, BROWN, BARNES, SCHMID, and SCHULTZ 99

Barnett, Henry L., Forman, Carolyn W., McNamara, Helen, McCrory, Wallace W., Rapoport, Milton, Michie, Alexander J., and **Barbero, Guilio.** The effect of adrenocorticotropic hormone on children with the nephrotic syndrome. II. Physiologic observations on discrete kidney functions and plasma volume 227

Barrett, E. See ADDIS, BARRETT, POO, UREEN, and LIPPMAN 206

Bartter, Frederic C., Albright, Fuller, Forbes, Anne P., Leaf, Alexander, Dempsey, Eleanor, and **Carroll, Evelyn.** The effects of adrenocorticotropic hormone and cortisone in the adrenogenital syndrome associated with congenital adrenal hyperplasia: an attempt to explain and correct its disordered hormonal pattern 237

—, and **Fourman, Paul.** The loss and the retention of potassium which occur immediately after starting and stopping adrenocorticotropic hormone, respectively 627

Bayles, Theodore B., Colpoys, Francis L., Fremont-Smith, Paul, Ferguson, Bruce C., and **Paige, Emil.** Significant remissions in rheumatic diseases with high dosage cortisone therapy 628

Bean, William Bennett, Franklin, Murray, Embick, James F., and Daum, Kate. Absorption studies using portal anastomotic veins 263
—. Clinical observations on the effects of adenylic acid and cozymase 628
—. See SCHROCK, SHEETS, and BEAN 174
—. See FRANKLIN, BEAN, PAUL, ROUTH, DE LA HUERGA, and POPPER 718
—. See FRANKLIN, BEAN, PAUL, ROUTH, DE LA HUERGA, and POPPER 729
Beck, William S. See VALENTINE and BECK 679
Becker, William H. See EICHNA, BERGER, RADER, and BECKER 353
Belle, M. S. See SCHEINBERG, JAYNE, RICH, and BELLE 671
Bennett, Ivan L., Jr., and Cluff, Leighton E. Studies on acquired resistance to the Shwartzman phenomenon 628
—. See MARTIN, BENNETT, and CHAUDHURI 659
Benson, John A., Jr. See GRAY, REIFENSTEIN, SPIRO, and BENSON 643
Bentinck, R. C., Gordan, Gilbert S., Adams, John E., Arnstein, L. H., and Leake, T. B. Effect of desoxycorticosterone glucoside upon cerebral blood flow and metabolism of human subjects 200
Berger, Adolph R. See EICHNA, BERGER, RADER, and BECKER 353
—. See EICHNA, FARBER, BERGER, EARLE, RADER, PELLEGRINO, ALBERT, ALEXANDER, TAUBE, and YOUNGWIRTH 1250
Berger, Eugene Y. See FARBER, BERGER, and EARLE 125
Berlin, N. I., Tolbert, B. M., and Lawrence, J. H. Studies in glycine-2-C^{14} metabolism in man. I. The pulmonary excretion of $C^{14}O_2$ 73
—. See PRENTICE, BERLIN, HYDE, PARSONS, LAWRENCE, and PORT 1471
Berry, John W. See GLASER, BERRY, LOEB, and WOOD 640
Best, William R., Muehrcke, Robert C., and Kark, Robert M. Studies on adrenocortical eosinopenia 629
Bethard, W. F., and Jacobson, L. O. Observations on survival of mice after exposure to lethal doses of X radiation 629
Bigelow, Frederick S. The influence of pH on blood coagulation 629
Bing, R. J., Falholt, W., Heimbecker, R., and Carroll, D. Myocardial oxidative metabolism and initial fiber length of the failing human heart 630
Birmingham, Marion K. See STERN, BIRMINGHAM, CULLEN, and RICHER 84
Bishop, Charles, Garner, William, and Talbott, John H. Pool size, turnover rate, and rapidity of equilibration of injected isotopic uric acid in normal and pathological subjects 879
—, **Rand, Royden, and Talbott, John H.** The effect of benemid (p-[di-n-propylsulfamyl]-benzoic acid) on uric acid metabolism in one normal and one gouty subject 889
Black, Harrison, and Roos, Albert. The effect of lipiodol on alveolar gas exchange 338
Blair, G. Walker. See HILDRETH, MELLINKOFF, BLAIR, and HILDRETH 649
Blakemore, A. H. See BRADLEY, MACPHERSON, GAMMELTOFT, and BLAKEMORE 630
—. See SMYTHE, FITZPATRICK, and BLAKEMORE 674
Bloom, Walter L. See WILSON, ESTES, DOYLE, and BLOOM 682
—. See WITHAM, FLEMING, and BLOOM 897
Bongiovanni, A. M. See KUNKEL, AHRENS, EISENMENGER, BONGIOVANNI, and SLATER 654
Bonner, C. D. See FISHMAN, SMITH, THOMPSON, BONNER, KASDON, and HOMBURGER 685
Bortolotti, Theresa R. See BUCKLEY and BORTOLOTTI 631
Boutourline-Young, H. J., and Whittenberger, J. L. The use of artificial respiration in pulmonary emphysema accompanied by high carbon dioxide levels 838
Bradley, S. E., Macpherson, A. I., Gammeltoft, A., and Blakemore, A. H. Effect of portacaval anastomosis on hepatic oxygen extraction and estimated hepatic blood flow in patients with cirrhosis 630
—. See CULBERTSON, WILKINS, INGELFINGER, and BRADLEY 305
Brandt, J. Leonard, Caccese, Anthony, Dock, William, and Schrager, Alvin. The motion of the thorax during the heart cycle: a comparison of longitudinal, lateral and dorsoventral ballistocardiograms 971
Brodie, Bernard B. See STOLLERMAN, BRODIE, LEVY, and POROSOWSKA 677
Brodsky, William A., and Rapoport, S. The mechanism of polyuria of diabetes insipidus in man. The effect of osmotic loading 282
Brofman, Bernard L. See CRAIG, MILLER, DRUCKER, WOODWARD, BROFMAN, and PRITCHARD 634
Brown, Charles Y. See HOLLANDER, BROWN, JESSAR, and BROWN 650
Brown, Ellen, Hopper, James, Jr., Sampson, John J., and Mudrick, Charles. Venous congestion of the extremities in relation to blood volume determinations and to mixing curves of carbon monoxide and T-1824 in normal human subjects 1441
Brown, Ernest M., Jr. See HOLLANDER, BROWN, JESSAR, and BROWN 650
—. See HOLLANDER, STONER, BROWN, and DEMOOR 701
Brown, G. Malcolm, Elliott, S. Margaret, and Young, W. A. The haemolytic factor in the anaemia of lymphatic leukaemia 130
Brown, Ray K. See LEVER, GURD, UROMA, BROWN, BARNES, SCHMID, and SCHULTZ 99
Brown, Thomas McP. See MERCHANT, BROWN, ROBINSON, and WICHELHAUSEN 660
Brownell, Gordon L. See STANBURY, GOLDSMITH, and BROWNELL 675
Brunschwig, Alexander. See TAGNON, SCHULMAN, BRUNSCHWIG, and LIEBERMAN 678
Brust, Albert A., Ransohoff, William, and Reiser, Morton F. Blood pressure responses to ACTH and cortisone in normotensive and hypertensive subjects in the resting state and during autonomic blockade with tetraethylammonium chloride 630
—, **Reiser, Morton F., and Ferris, Eugene B., Jr.** Evaluation of neurogenic control of blood pressure in hypertension with tetraethylammonium chloride and spinal anesthesia 925
Buchanan, Donald L. Elimination of intravenous carbon^{14} bicarbonate by man 630
Buckley, Edward S., Jr., and Bortolotti, Theresa R. The determination of traces of calcium and magnesium in plasma 631
Buechler, Peter R. See WEISS, SILER, and BUECHLER 1507
Bukantz, Samuel C. See DAMMIN, BUKANTZ, DIXON, and TALMAGE 635

Bunn, Paul A., and **Canarili, Leonard.** Combined antimicrobial action upon penicillin resistant organisms *in vitro* 631
Burchenal, Joseph H., and **Kingsley-Pillers, E. M.** Studies on amethopterin, citrovorum factor, and crude X-methyl folic acid in leukemia 631
Burnell, James M., and **Kirby, William M. M.** Effectiveness of a new compound, Benemid, in elevating serum penicillin concentrations 697
Burton, Robert B., Zaffaroni, Alejandro, Izzo, Anthony J., and **Keutmann, E. Henry.** Studies on the nature of the adrenal cortical secretion in patients with malignancy 632

C

Cabieses, Fernando. See SHENKIN, CABIESES, and VAN DEN NOORDT 90
Caccese, Anthony. See BRANDT, CACCESE, DOCK, and SCHRAGER 971
Callahan, Edmund J. See STEARNS and CALLAHAN 676
Campbell, James A., Selverstone, Louis A., and **Donovan, Daniel L.** Studies on the high output cardiac failure of occidental beriberi 632
Canarili, Leonard. See BUNN and CANARILI 631
Cannon, John L. See PATTERSON and CANNON 664
Carbone, John. See KINSELL, MARGEN, MICHAELS, REISS, FRANTZ, and CARBONE 1491
Carroll, D. See BING, FALHOLT, HEIMBECKER, and CARROLL 630
Carroll, Evelyn. See BARTTER, ALBRIGHT, FORBES, LEAF, DEMPSEY, and CARROLL 237
Cartwright, G. E., Hamilton, L. D., Gubler, C. J., Fellows, N. M., Ashenbrucker, Helen, and **Wintrobe, M. M.** The anemia of infection. XIII. Studies on experimentally produced acute hypoferremia in dogs and the relationship of the adrenal cortex to hypoferremia 161
Cayle, Theodore. See ALBAUM, CAYLE, and SHAPIRO 525
Chapman, Don W., and **Ory, Alan A.** Effects of paritol on the prothrombin times, antithrombin times, and Lee-White clotting times 632
Chasen, William C. See LEMON, LOONEY, and CHASEN 655
Chatterjea, Jyoti B. See STEFANINI and CHATTERJEA 676
Chaudhuri, S. See MARTIN, BENNETT, and CHAUDHURI 659
Childs, Barton. See NAJJAR and CHILDS 663
Christian, Richard M. See SAMSON, SWISHER, CHRISTIAN, and ENGEL 669
Clark, John Kapp, Crosley, Archer P., Jr., and **Barker, Harold G.** Effect of subtotal adrenalectomy on renal hemodynamics and electrolyte excretion in human hypertension 633
—, and **Barker, Harold G.** Studies of renal oxygen consumption in man. I. The effect of tubular loading (PAH), water diuresis and osmotic (mannitol) diuresis 745
—. See SINGER, CLARK, and CROSLEY 674
Clark, William S., Manning, Elizabeth L., and **Appleton, Janet E.** Observations on the metabolic and anti-inflammatory effects of desoxycorticosterone, testosterone and cortisone in a patient with rheumatoid arthritis 633
Clarke, C. E. See PETERS, DANOWSKI, GREENMAN, WEIGAND, CLARKE, GARVER, MATEER, and TARAIL 1009
Cleroux, A. P. See PAYNE, ROSENBERG, RABEN, CLEROUX, and ASTWOOD 665
Cluff, Leighton E. See BENNETT and CLUFF 628
Coates, E. Osborne, and **Comroe, Julius H., Jr.** Pulmonary function studies in sarcoidosis 848
Cockrell, Marion S. See HUFF, ELMLINGER, GARCIA, ODA, and COCKRELL 651
—. See HUFF, ELMLINGER, GARCIA, ODA, COCKRELL, and LAWRENCE 1512
Coith, C. L. See ALTEMEIER, COITH, CULBERTSON, LOGAN, and TYTELL 627
Colpoys, Francis L. See BAYLES, COLPOYS, FREMONT-SMITH, FERGUSON, and PAIGE 628
Comfort, Mandred W. See TAYLOR, COMFORT, WOLLAEGER, and POWER 678
Comroe, Julius H., Jr. See COATES and COMROE 848
Conley, C. Lockard. See HARTMANN, CONLEY, and KREVANS 647
—. See HARTMANN, CONLEY, and KREVANS 948
Cook, C. D. See ALEXANDER, GOLDSTEIN, LANDWEHR, and COOK 596
Cooper, Gerald R. See RUNDLES, COOPER, and WILLETT 669
—. See RUNDLES, COOPER, and WILLETT 1125
Corcoran, A. C., Dustan, Harriet P., and **Page, Irvine H.** Enzymatically hydrolyzable formaldehydogenic corticoids: normal values and observations on disease states 633
Corley, R. W. See HOOBLER, CORLEY, and KABZA 651
Cort, Joseph H. See SELDIN, WELT, and CORT 673
—. See WELT, SELDIN, and CORT 682
Cotzias, George C. See DOLE, DAHL, COTZIAS, DZIEWIATKOWSKI, and HARRIS 584
Coulter, M. See HOWLAND, FURTH, and COULTER 651
Craig, James W., Miller, Max, Drucker, William R., Woodward, Hiram, Brofman, Bernard L., and **Pritchard, Walter H.** *In vivo* studies of the role of the kidney in intermediary metabolism in man and dog 634
Craig, Lyman C. See AHRENS and CRAIG 626
Crawford, Betty, and **Ludemann, Henry.** The renal response to intravenous injections of sodium chloride solutions in man 1456
Crockett, C. L., Jr. See ROSS, CROCKETT, and EMERSON 668
Crosley, Archer P., Jr. See ELKINTON, SQUIRES, and CROSLEY 369
—. See CLARK, CROSLEY, and BARKER 633
—. See SINGER, CLARK, and CROSLEY 674
Crumpton, Charles W., Friedland, Carl K., Kuo, Peter T., Schnabel, Truman G., Jr., Foltz, Elwood L., Page, Robert G., Alanis, Jesus, and **Hafkenschiel, Joseph H.** The hemodynamic actions of protoveratrine in essential hypertension 634
—. See HAFKENSCHIEL, CRUMPTON, SHENKIN, MOYER, ZINTEL, WENDEL, and JEFFERS 793
Cubiles, Ricardo. See SCHMIDT, FULD, CUBILES, and PROGER 671
Cugell, David W. See GAENSLER and CUGELL 639
Culbertson, James W., Wilkins, Robert W., Ingelfinger, Franz J., and **Bradley, Stanley E.** The effect of the upright posture upon hepatic blood flow in normotensive and hypertensive subjects 305
—. See WILKINS, CULBERTSON, and INGELFINGER 312
Culbertson, W. R. See ALTEMEIER, COITH, CULBERTSON, LOGAN, and TYTELL 627
Cullen, Andrea. See STERN, BIRMINGHAM, CULLEN, and RICHER 84

Currie, Gordon D. See WOLF, REMP, KILEY, and CURRIE 683
—. See WOLF, REMP, KILEY, and CURRIE 1062

D

Dahl, Lewis K. See DOLE, DAHL, COTZIAS, DZIEWIATKOWSKI, and HARRIS 584
Dammin, Gustave J., Bukantz, Samuel C., Dixon, Frank J., and **Talmage, David W.** The metabolism of I^{131} labelled proteins in rabbits: the sensitivity of the antigen elimination rate as an indicator of antibody response 635
Danowski, T. S., Greenman, L., Peters, J. H., and **Mateer, F. M.** An introduction to experimental and clinical studies of carboxylic cation exchange resins 979
—, **Greenman, L., Mateer, F. M., Parsons, W. B., Weigand, F. A., Mermelstein, H.,** and **Peters, J. H.** Carboxylic cation exchange resin effects in dogs 984
—. See GREENMAN, DANOWSKI, TARAIL, PETERS, WEIGAND, and MATEER 644
—. See GREENMAN, PETERS, MATEER, WEIGAND, WILKINS, TARAIL, RHODES, and DANOWSKI 995
—. See PETERS, DANOWSKI, GREENMAN, WEIGAND, CLARKE, GARVER, MATEER, and TARAIL 1009
—. See MATEER, ERHARD, PRICE, WEIGAND, PETERS, DANOWSKI, TARAIL, and GREENMAN 1018
—. See GREENMAN, PETERS, MATEER, and DANOWSKI 1027
Daughaday, William H., and **Farr, A. Lewis.** Adrenal cortical reserve in severe hyperthyroidism 635
Daum, Kate. See BEAN, FRANKLIN, EMBICK, and DAUM 263
Davidson, Charles S. See GABUZDA, PHILLIPS, SCHILLING, and DAVIDSON 639
Davis, Bernard D. Use of mutants to reveal a vitamin (POB) antagonizing the chemotherapeutic action of PABA 635
Davis, Robert K. See ROSENBAUM, DAVIS, and FERGUSON 668
—. See STRAUSS, DAVIS, ROSENBAUM, and ROSSMEISL 862
Dayman, Howard G. Measurement of pulmonary pressure 635
—. Mechanics of airflow in health and in emphysema 1175
Deane, Norman, Schreiner, George E., and **Robertson, James S.** The velocity of distribution of sucrose between plasma and interstitial fluid, with reference to the use of sucrose for the measurement of extracellular fluid in man 1463
—. Intracellular water in man 1469
DeGowin, Elmer L. See SHEETS, JANNEY, HAMILTON, and DEGOWIN 1272
de la Huerga, Jesus, and **Popper, Hans.** Urinary excretion of choline metabolites following choline administration in normals and patients with hepatobiliary diseases 463
—. See FRANKLIN, BEAN, PAUL, ROUTH, DE LA HUERGA, and POPPER 718
—. See FRANKLIN, BEAN, PAUL, ROUTH, DE LA HUERGA, and POPPER 729
Deming, Quentin B. See LUETSCHER, DEMING, and JOHNSON 658
—. See LUETSCHER, DEMING, and JOHNSON 1530
deMoor, Peter. See HOLLANDER, STONER, BROWN, and DEMOOR 701
Dempsey, Eleanor. See BARTTER, ALBRIGHT, FORBES, LEAF, DEMPSEY, and CARROLL 237
Denny, Floyd W. See HAHN, HOUSER, RAMMELKAMP, DENNY, and WANNAMAKER 274
Desforges, J. F. See HARRIS, JORDAN, PILLEMER, and DESFORGES 646
Dexter, Lewis. See GORLIN, DEXTER, and HARKEN 643
Di Caprio, Joseph M. See RANTZ and DI CAPRIO 667
Dingle, John H. See JORDAN, PILLEMER, and DINGLE 11
—. See JORDAN, PILLEMER, and DINGLE 22
Dixon, Frank J. See DAMMIN, BUKANTZ, DIXON, and TALMAGE 635
Dobyns, Brown M., and **Lennon, Beatrice.** The behavior of human thyroid tumors transplanted to the anterior chamber of guinea pig eyes 636
Dock, William. See BRANDT, CACCESE, DOCK, and SCHRAGER 971
Dohan, Francis C. See JEFFERS, ZINTEL, HAFKENSCHIEL, DOHAN, and KEFFER 652
Dole, Vincent P., Dahl, Lewis K., Cotzias, George C., Dziewiatkowski, Dominic D., and **Harris, Cecilia.** Dietary treatment of hypertension. II. Sodium depletion as related to the therapeutic effect 584
Donovan, Daniel L. See CAMPBELL, SELVERSTONE, and DONOVAN 632
Douglass, R. A. See ASSALI, KAPLAN, FOMON, DOUGLASS, and TADA 916
Doyle, Joseph T., Wilson, Joseph S., Estes, E. Harvey, and **Warren, James V.** The effect of intravenous infusions of physiologic saline solution on the pulmonary arterial and pulmonary capillary pressure in man 345
—. See WARREN, DOYLE, ESTES, and WILSON 680
—. See WILSON, ESTES, DOYLE, and BLOOM 682
Drachman, Stanley R. See ADLERSBERG, DRACHMAN, SCHAEFER, and DRITCH 626
Dripps, Robert D. See PRICE, KING, ELDER, LIBIEN, and DRIPPS 1243
Dritch, Rhoda. See ADLERSBERG, DRACHMAN, SCHAEFER, and DRITCH 626
Drucker, William R. See CRAIG, MILLER, DRUCKER, WOODWARD, BROFMAN, and PRITCHARD 634
Dubois, Edmund L. Acquired hemolytic anemia as the presenting syndrome of lupus erythematosus disseminata 636
Duke, T. Whatley. See PATTERSON, HEYMAN, and DUKE 665
Duncan, Leroy E., Jr., Solomon, David H., Nichols, Mary P., and **Rosenberg, Esther.** The effect of the chronic administration of adrenal medullary hormones to man on adrenocortical function and the renal excretion of electrolytes 908
Dustan, Harriet P. See CORCORAN, DUSTAN, and PAGE 633
Dziewiatkowski, Dominic D. See DOLE, DAHL, COTZIAS, DZIEWIATKOWSKI, and HARRIS 584

E

Earle, D. P., Farber, S. J., Alexander, J. D., and **Pellegrino, E. D.** Renal function and electrolyte metabolism in acute glomerulonephritis 421
—. See FARBER, BERGER, and EARLE 125

—. See EICHNA, FARBER, BERGER, EARLE, RADER, PELLEGRINO, ALBERT, ALEXANDER, TAUBE, and YOUNGWIRTH 1250
Ebert, R. H. Changes in connective tissue reaction induced by cortisone—*in vivo* observations using the rabbit ear chamber technique 636
Eckstein, R. W. See PRITCHARD, MACINTYRE, ECKSTEIN, and FRIEDELL 666
Eddy, Walter H. See SOKOLOFF, EDDY, and REDD 395
Edelman, I. S., and **Moore, F. D.** Body water, water distribution, and water kinetics as revealed by the use of deuterium oxide 637
Edwards, Clifford J. See HINKLE, EDWARDS, and WOLF 809
—. See HINKLE, EDWARDS, and WOLF 818
Eichelberger, Lillian. See RICKETTS, EICHELBERGER, and KIRSNER 1157
Eichna, Ludwig W., Berger, Adolph R., Rader, Bertha, and **Becker, William H.** Comparison of intracardiac and intravascular temperatures with rectal temperatures in man 353
—, **Farber, Saul J., Berger, Adolph R., Earle, David P., Rader, Bertha, Pellegrino, Edmund, Albert, Roy E., Alexander, J. Deaver, Taube, Harry,** and **Youngwirth, Sol.** The interrelationships of the cardiovascular, renal and electrolyte effects of intravenous digoxin in congestive heart failure 1250
—. See FARBER, ALEXANDER, and EICHNA 638
Eidinoff, Maxwell. See HELLMAN, PEACOCK, EIDINOFF, ROSENFELD, and GALLAGHER 648
Einbinder, Julia. See SCHUBERT and EINBINDER 673
Eisenmenger, W. J. See KUNKEL, AHRENS, EISENMENGER, BONGIOVANNI, and SLATER 654
Elder, John D. See PRICE, KING, ELDER, LIBIEN, and DRIPPS 1243
Eliel, Leonard P., Pearson, Olof H., and **West, Charles D.** Excessive losses of sodium, chloride, and water after administration of cortisone or ACTH, suggesting transient adrenocortical insufficiency 637
—. See PEARSON, ELIEL, and HOLLANDER 665
Elkin, Daniel C. See WARREN, NICKERSON, and ELKIN 210
—. See NICKERSON, ELKIN, and WARREN 215
—. See WARREN, ELKIN, and NICKERSON 220
Elkinton, J. R., Squires, R. D., and **Crosley, A. P., Jr.** Intracellular cation exchanges in metabolic alkalosis 369
—, **Squires, R. D.,** and **Singer, R. B.** Intracellular cation exchanges in acidosis due to renal insufficiency 381
—. See SQUIRES and ELKINTON 675
Elliott, S. Margaret. See BROWN, ELLIOTT, and YOUNG 130
Ellis, Frank W., and **West, Philip M.** Sensitivity of serum enzyme inhibitors to a variety of cancer chemotherapeutic agents 547
Elmlinger, Paul J. See HUFF, ELMLINGER, GARCIA, ODA, and COCKRELL 651
—. See HUFF, ELMLINGER, GARCIA, ODA, COCKRELL, and LAWRENCE 1512
Embick, James F. See BEAN, FRANKLIN, EMBICK, and DAUM 263
Emerson, C. P. See ROSS, CROCKETT, and EMERSON 668
Emerson, Kendall, Jr., Roche, Marcel, Kahn, Stanley S., Moser, Hugo W., and **Jenkins, Dalton.** Observations on the mechanism of the hyperlipemia in the nephrotic syndrome 637
Engel, George L. See SAMSON, SWISHER, CHRISTIAN, and ENGEL 669
Engstrom, William W., Kydd, David M., Peters, John P., and **Man, Evelyn B.** The precipitable iodine of serum in pregnancy complicated by disorders of the thyroid 151
Epstein, Franklin H., Goodyer, Allan V. N., Lawrason, F. Douglas, and **Relman, Arnold S.** Studies of the antidiuresis of quiet standing: the importance of changes in plasma volume and glomerular filtration rate 63
Erhard, Loraine H. See MATEER, ERHARD, PRICE, WEIGAND, PETERS, DANOWSKI, TARAIL, and GREENMAN 1018
Estes, E. Harvey. See DOYLE, WILSON, ESTES, and WARREN 345
—. See WARREN, DOYLE, ESTES, and WILSON 680
—. See WILSON, ESTES, DOYLE, and BLOOM 682
Evans, Everett I. See JAMES, PURNELL, and EVANS 181
—. See JAMES, PURNELL, and EVANS 191

F

Falholt, W. See BING, FALHOLT, HEIMBECKER, and CARROLL 630
Faloon, William W., Greene, Richard W., and **Lozner, Eugene L.** The hemostatic defect in thrombocytopenia as studied by the use of ACTH and cortisone 638
Farber, Saul J., Berger, Eugene Y., and **Earle, David P.** Effect of diabetes and insulin on the maximum capacity of the renal tubules to reabsorb glucose 125
—, **Alexander, J. Deaver,** and **Eichna, Ludwig W.** Renal hemodynamics and salt and water excretion during induced congestion of the inferior vena cava of man 638
—. See EARLE, FARBER, ALEXANDER, and PELLEGRINO 421
—. See EICHNA, FARBER, BERGER, EARLE, RADER, PELLEGRINO, ALBERT, ALEXANDER, TAUBE, and YOUNGWIRTH 1250
Farr, A. Lewis. See DAUGHADAY and FARR 635
Fellows, N. M. See CARTWRIGHT, HAMILTON, GUBLER, FELLOWS, ASHENBRUCKER, and WINTROBE 161
Fenninger, Leonard D. See WATERHOUSE, KEUTMANN, and FENNINGER 681
Ferguson, Bruce C. See BAYLES, COLPOYS, FREMONT-SMITH, FERGUSON, and PAIGE 628
—. See ROSENBAUM, DAVIS, and FERGUSON 668
Ferguson, John H. See TRAVIS and FERGUSON 112
Ferrebee, Joseph W. See GARDELLA and FERREBEE 640
Ferris, Eugene B., Jr. An inquiry into the meaning of clinical investigation 623
—. See BRUST, REISER, and FERRIS 925
Finberg, Laurence. See HARRISON, FINBERG, and FLEISHMAN 646
Finnerty, Frank A., Jr. See FREIS, STANTON, FINNERTY, SCHNAPER, JOHNSON, RATH, and WILKINS 435
Fishman, W. H., Smith, M., Thompson, D. B., Bonner, C. D., Kasdon, S. C., and **Homburger, F.** Investigation of glucuronic acid metabolism in human subjects 685
Fitzpatrick, H. F. See SMYTHE, FITZPATRICK, and BLAKEMORE 674
Fleishman, Evelyn. See HARRISON, FINBERG, and FLEISHMAN 646
Fleming, J. W. See WITHAM and FLEMING 707
—. See WITHAM, FLEMING, and BLOOM 897

Foltz, Elwood L. See CRUMPTON, FRIEDLAND, KUO, SCHNABEL, FOLTZ, PAGE, ALANIS, and HAFKENSCHIEL 634
Fomon, S. J. See ASSALI, KAPLAN, FOMON, DOUGLASS, and TADA 916
Forbes, Anne P. See BARTTER, ALBRIGHT, FORBES, LEAF, DEMPSEY, and CARROLL 237
Forbes, Gilbert B., and **Perley, Anne.** Estimation of total body sodium by isotopic dilution. I. Studies on young adults 558
—, and —. Estimation of total body sodium by isotopic dilution. II. Studies on infants and children; an example of a constant differential growth ratio 566
Forman, Carolyn W. See BARNETT, FORMAN, MCNAMARA, MCCRORY, RAPOPORT, MICHIE, and BARBERO 227
Forsham, Peter H. See RENOLD, MAISTEREENA, and FORSHAM 667
Fourman, Paul. See BARTTER and FOURMAN 627
Fowler, Noble O., Westcott, Richard N., Scott, Ralph C., and **McGuire, Johnson.** The effect of nor-epinephrine upon pulmonary arteriolar resistance in man 517
—. See WESTCOTT, FOWLER, SCOTT, HAUENSTEIN, and MCGUIRE 957
Frank, Heddy. See KRAMER, MINKEL, FRANK, and INGELFINGER 653
Franklin, Murray, Bean, William B., Paul, William D., Routh, J. I., de la Huerga, Jesus, and **Popper, Hans.** Electrophoretic studies in liver disease. I. Comparison of serum and plasma electrophoretic patterns in liver disease, with special reference to fibrinogen and gamma globulin patterns 718
—, **Bean, William B., Paul, William D., Routh, J. I., de la Huerga, Jesus,** and **Popper, Hans.** Electrophoretic studies in liver disease. II. Gamma$_1$ globulin in chronic liver disease 729
—. See BEAN, FRANKLIN, EMBICK, and DAUM 263
Frantz, Robert. See KINSELL, MARGEN, MICHAELS, REISS, FRANTZ, and CARBONE 1491
Frawley, Thomas F., Roche, Marcel, Jenkins, Dalton, and **Thorn, George W.** The role of the pituitary adrenocortical system in the response to anoxia 638
Freed, S. Charles. See FRIEDMAN, FREED, and ROSEMAN 639
Freis, Edward D., Stanton, Joseph R., Finnerty, Frank A., Jr., Schnaper, Harold W., Johnson, Robert L., Rath, Charles E., and **Wilkins, Robert W.** The collapse produced by venous congestion of the extremities or by venesection following certain hypotensive agents 435
—. See SCHNAPER, JOHNSON, TUOHY, and FREIS 786
Fremont-Smith, Paul. See BAYLES, COLPOYS, FREMONT-SMITH, FERGUSON, and PAIGE 628
Friedell, H. L. See PRITCHARD, MACINTYRE, ECKSTEIN, and FRIEDELL 666
Friedland, Carl K. See CRUMPTON, FRIEDLAND, KUO, SCHNABEL, FOLTZ, PAGE, ALANIS, and HAFKENSCHIEL 634
Friedman, Meyer, Freed, S. Charles, and **Roseman, Ray H.** Reduction of blood pressure in the normotensive and hypertensive animal following potassium deprivation 639
Fuld, Maria. See SCHMIDT, FULD, CUBILES, and PROGER 671
Furth, F. W. See HOWLAND, FURTH, and COULTER 651

G

Gabuzda, George J., Jr., Phillips, Gerald B., Schilling, Robert F., and **Davidson, Charles S.** Metabolism of pteroylglutamic acid and citrovorum factor in human scurvy 639
Gaensler, Edward A. Quantitative determination of the visceral pain threshold in man 406
—, and **Cugell, David W.** Ambient air recording bronchospirometry and differential residual volume determination 639
Gallagher, Thomas. See HELLMAN, PEACOCK, EIDINOFF, ROSENFELD, and GALLAGHER 648
Gammeltoft, A. See BRADLEY, MACPHERSON, GAMMELTOFT, and BLAKEMORE 630
Ganong, Frank. See LOWN and GANONG 658
Garcia, Joseph F. See HUFF, ELMLINGER, GARCIA, ODA, and COCKRELL 651
—. See HUFF, ELMLINGER, GARCIA, ODA, COCKRELL, and LAWRENCE 1512
Gardella, Joseph W., and **Ferrebee, Joseph W.** Observations on insulin labeled with radio iodine 640
Gardner, Frank H. See HIRSCH and GARDNER 649
—. See LEY and GARDNER 656
Garner, William. See BISHOP, GARNER, and TALBOTT 879
Garson, Warfield. See MAGNUSON, TAUBER, MCLEOD, and GARSON 659
Garver, K. See PETERS, DANOWSKI, GREENMAN, WEIGAND, CLARKE, GARVER, MATEER, and TARAIL 1009
Gauer, Otto H. See HENRY, GAUER, KETY, and KRAMER 292
Geyer, Robert P. See VAN ITALLIE, GEYER, and STARE 680
Gilbert, Robert P., Aldrich, Stephen L., and **Anderson, Lynn.** Cardiac output in acute myocardial infarction 640
Ginsberg, H. S., and **Horsfall, F. L., Jr.** Mechanism of production of lung lesions in virus pneumonia 640
Glaser, Robert J., Berry, John W., Loeb, Lenore H., and **Wood, W. Barry, Jr.** The effect of cortisone on acute bacterial infections 640
Glendening, Mary Beth, and **Page, Ernest W.** The site of inhibition of blood clotting by soy bean trypsin inhibitor 1298
Goldberg, H., Silber, E. N., and **Gordon, A.** Dynamics of Eisenmenger's complex 641
—. See SILBER, SNIDER, GOLDBERG, KATZ, and RADNER 1046
Goldenberg, Marcel, and **Rapport, Maurice M.** Norepinephrine and epinephrine in human urine (Addison's disease, essential hypertension, pheochromocytoma) 641
Goldman, Ralph. Studies in diurnal variation of water and electrolytes: nocturnal diuresis of water and sodium in congestive cardiac failure, cirrhosis of the liver and degenerative glomerulonephritis 642
—. Studies in diurnal variation of water and electrolyte excretion: nocturnal diuresis of water and sodium in congestive cardiac failure and cirrhosis of the liver 1191
Goldsmith, Richard E. See STANBURY, GOLDSMITH, and BROWNELL 675
Goldstein, Robert. See ALEXANDER, GOLDSTEIN, and LANDWEHR 252
—. See ALEXANDER, GOLDSTEIN, LANDWEHR, and COOK 596

—. See ALEXANDER, GOLDSTEIN, and LANDWEHR 627
Goldwater, William H. See SNAVELY, GOLDWATER, RANDOLPH, SPRAGUE, and UNGLAUB 674
—. See TURNER, SNAVELY, GOLDWATER, RANDOLPH, SPRAGUE, and UNGLAUB 1071
Gómez, Domingo M. Evaluation of renal resistances with special reference to changes in essential hypertension 1143
Good, Robert A. See THOMAS, MOGABGAB, and GOOD 678
Goodale, Walter T., Olson, Robert E., and **Hackel, Donald B.** The effect of mild diabetes and insulin on heart muscle metabolism in man 642
—. See MERRILL, GOODALE, and SWAN 660
Goodell, Helen. See GRAHAM, GOODELL, and WOLFF 37
Goodyer, A. V. N., and **Seldin, D. W.** The effects of quiet standing on soluble diuresis 642
—. See EPSTEIN, GOODYER, LAWRASON, and RELMAN 63
—. See TARAIL, SELDIN, and GOODYER 1111
Gordan, Gilbert S. See BENTINCK, GORDAN, ADAMS, ARNSTEIN, and LEAKE 200
Gordon, A. See GOLDBERG, SILBER, and GORDON 641
Gorlin, Richard, Dexter, Lewis, and **Harken, Dwight E.** Circulatory changes in mitral stenosis following valvuloplasty 643
Gottsegen, Robert. See GROKOEST, VAILLANCOURT, GOTTSEGEN, and RAGAN 644
Grace, William J., and **Graham, David T.** The specificity of the relation between attitudes and diseases 643
Graham, David T., Goodell, Helen, and **Wolff, Harold G.** Neural mechanisms involved in itch, "itchy skin," and tickle sensations 37
—. See GRACE and GRAHAM 643
Gray, Seymour J., Reifenstein, Robert W., Spiro, Howard M., and **Benson, John A., Jr.** Evidence for an adrenal phase of gastric secretion 643
Greene, Richard W. See FALOON, GREENE, and LOZNER 638
Greenman, L., Danowski, T. S , Tarail, R., Peters, J. H., Weigand, F., and **Mateer, F.** Studies of prevention of some of the toxic effects of prolonged cortisone and ACTH therapy 644
—, **Peters, J. H., Mateer, F. M., Weigand, F. A., Wilkins, D., Tarail, R., Rhodes, G.,** and **Danowski, T. S.** Biochemical changes accompanying the ingestion of a carboxylic cation exchanger in the hydrogen, ammonium, sodium, potassium, or calcium form 995
—, **Peters, J. H., Mateer, F. M.,** and **Danowski, T. S.** Probable clinical utility of cation exchange resins 1027
—. See DANOWSKI, GREENMAN, PETERS, and MATEER 979
—. See DANOWSKI, GREENMAN, MATEER, PARSONS, WEIGAND, MERMELSTEIN, and PETERS 984
—. See PETERS, DANOWSKI, GREENMAN, WEIGAND, CLARKE, GARVER, MATEER, and TARAIL 1009
—. See MATEER, ERHARD, PRICE, WEIGAND, PETERS, DANOWSKI, TARAIL, and GREENMAN 1018
Greer, Monte A. Correlation of the 24-hour radioiodine uptake of the human thyroid gland with the six- and eight-hour uptakes and the "accumulation gradient" 301
—. The results of feeding desiccated thyroid to thyrotoxic subjects 644
Grokoest, Albert W., Vaillancourt, deGuise, Gottsegen, Robert, and **Ragan, Charles.** Lack of analgesic effect of cortisone and ACTH 644
Grollman, Arthur, Turner, Louis B., and **Muirhead, E. E.** A study of the hemodynamic and pathological changes induced by bilateral nephrectomy and ligation of the ureters of dogs maintained for prolonged periods by means of intermittent peritoneal lavage 644
Grossman, J., and **Weston, R. E.** Determination of cardiac output in man by the Fick principle without gas analysis 645
—, **Weston, R. E., Lehman, R. A., Halperin, J. P., Ullmann, T. D.,** and **Leiter, L.** Urinary and fecal excretion of mercury in man following administration of mercurial diuretics 1208
—. See WESTON, GROSSMAN, LEHMAN, ULLMANN, HALPERIN, and LEITER 1221
—. See WESTON, GROSSMAN, and LEITER 1262
Gubler, C. J. See CARTWRIGHT, HAMILTON, GUBLER, FELLOWS, ASHENBRUCKER, and WINTROBE 161
Gunn, F. D. See TYLER, STEPHENS, GUNN, and PERKOFF 492
Gurd, Frank R. N. See LEVER, GURD, UROMA, BROWN, BARNES, SCHMID, and SCHULTZ 99

H

Hackel, Donald B. See GOODALE, OLSON, and HACKEL 642
Hafkenschiel, Joseph H., Crumpton, Charles W., Shenkin, Henry A., Moyer, John H., Zintel, Harold A., Wendel, Herbert, and **Jeffers, William A.** The effects of 20° head-up tilt upon the cerebral circulation of patients with arterial hypertension before and after sympathectomy 793
—. See CRUMPTON, FRIEDLAND, KUO, SCHNABEL, FOLTZ, PAGE, ALANIS, and HAFKENSCHIEL 634
—. See JEFFERS, ZINTEL, HAFKENSCHIEL, DOHAN, and KEFFER 652
Hahn, Edward O., Houser, Harold B., Rammelkamp, Charles H., Jr., Denny, Floyd W., and **Wannamaker, Lewis W.** Effect of cortisone on acute streptococcal infections and post-streptococcal complications 274
Hall, H. E. See WOODWARD, HALL, HIGHTOWER, and PONS 683
Hall, Wendell H., and **Manion, Robert E.** Hemagglutinins and hemolysins for erythrocytes sensitized with tuberculin in pulmonary tuberculosis 1542
Halperin, J. P. See GROSSMAN, WESTON, LEHMAN, HALPERIN, ULLMANN, and LEITER 1208
—. See WESTON, GROSSMAN, LEHMAN, ULLMANN, HALPERIN, and LEITER 1221
Hamilton, Henry E. See SHEETS, JANNEY, HAMILTON, and DEGOWIN 1272
Hamilton, L. D. See CARTWRIGHT, HAMILTON, GUBLER, FELLOWS, ASHENBRUCKER, and WINTROBE 161
Hammarsten, J. F. See JACOBSON, HAMMARSTEN, and HELLER 1503
Harken, Dwight E. See GORLIN, DEXTER, and HARKEN 643
Harrell, George T., Masland, Richard L., and **Rosenblum, Marvin J.** Central nervous system sequelae as shown by post-recovery electroencephalograms in rickettsial spotted fever 645
—. See AIKAWA and HARRELL 360
—. See AIKAWA, HARRELL, and MILLER 575
Harrington, William J., Hollingsworth, J. William, Minnich, Virginia, and **Moore, Carl V.** Demonstration of a thrombocytopenic factor in the blood of patients with idiopathic thrombocytopenic purpura 646

Harris, Cecilia. See DOLE, DAHL, COTZIAS, DZIEWIATKOWSKI, and HARRIS 584
Harris, J. F. See LLOYD, HARRIS, and LOBOTSKY 657
Harris, J. W., Jordan, W. S., Pillemer, L., and Desforges, J. F. The enzymatic nature of the factor in normal serum which hemolyses the erythrocytes of paroxysmal nocturnal hemoglobinuria 646
—. See KASS, INGBAR, HARRIS, and LEY 653
Harrison, Harold E., Finberg, Laurence, and Fleishman, Evelyn. Disturbances of concentrations of intracellular and extracellular electrolytes in patients with tuberculous meningitis 646
Hartmann, Robert C., Conley, C. Lockard, and Krevans, Julius R. Studies on "heparin tolerance": an analysis of factors influencing the anticoagulant activity of heparin 647
—, **Conley, C. Lockard, and Krevans, Julius R.** The effect of intravenous infusion of thromboplastin on "heparin tolerance" 948
Hauenstein, Virgil D. See WESTCOTT, FOWLER, SCOTT, HAUENSTEIN, and MCGUIRE 957
Havens, W. Paul, Jr., Shaffer, James M., and Hopke, Charles J., Jr. The capacity of patients with chronic hepatic disease to produce antibody, and the effect of ACTH and cortisone on this function 647
Haynes, Florence W. See LEWIS, HAYNES, and SPIEGL 656
Hecht, Hans H., Osher, William J., and Samuels, Arthur J. Cardiovascular adjustments in subjects with organic heart disease before and after conversion of atrial fibrillation to normal sinus rhythm 647
Heimbecker, R. See BING, FALHOLT, HEIMBECKER, and CARROLL 630
Heinemann, Martin. See MAN, HEINEMANN, JOHNSON, LEARY, and PETERS 137
—. See PETERS, HEINEMANN, and MAN 388
Heinle, Robert W. See WEISBERGER, HEINLE, and LEVINE 681
Heller, B. I. See JACOBSON, HAMMARSTEN, and HELLER 1503
Heller, Carl G., Nelson, Warren O., Maddock, William O., Jungck, Edwin C., Paulsen, C. Alvin, and Mortimore, Glenn E. Effect of testosterone upon the human testis 648
—. See RIGAS and HELLER 853
Hellman, Leon, Peacock, Wendell, Eidinoff, Maxwell, Rosenfeld, Robert, and Gallagher, Thomas. The metabolic fate of radioactive carbon labelled acetate and glycine in humans 648
—. See RALL, HELLMAN, PEACOCK, and RAWSON 667
Henry, James P., Gauer, Otto H., Kety, Seymour S., and Kramer, Kurt. Factors maintaining cerebral circulation during gravitational stress 292
Hess, Eugene L. See AYALA, MOORE, and HESS 781
Heyman, Albert. See PATTERSON, HEYMAN, and DUKE 665
Hickam, J. B., and Pryor, W. W. Cardiac output in postural hypotension 401
—, **Pryor, W. W., Page, E. B., and Atwell, R. J.** Respiratory regulation during exercise in unconditioned subjects 503
—, **Sieker, H. O., and Ryan, J. M.** Hypoxia as a respiratory stimulant in cardiac dyspnea 648
Hightower, J. A. See WOODWARD, HALL, HIGHTOWER, and PONS 683
Hildreth, Dorothy M. See HILDRETH, MELLINKOFF, BLAIR, and HILDRETH 649
Hildreth, Eugene A., Mellinkoff, Sherman M., Blair, G. Walker, and Hildreth, Dorothy M. An experimental study of practical diets to reduce the human serum cholesterol 649
Hilton, James G. Potentiation of diuretic action of Mercuhydrin by ammonium chloride 1105
Hinkle, Lawrence E., Jr., and Wolf, Stewart. Variations in blood glucose in diabetes mellitus 649
—, **Edwards, Clifford J., and Wolf, Stewart.** The occurrence of diuresis in humans in stressful situations and its possible relation to the diuresis of early starvation 809
—, **Edwards, Clifford J., and Wolf, Stewart.** Studies in diabetes mellitus. II. The occurrence of a diuresis in diabetic persons exposed to stressful life situations, with experimental observations on its relation to the concentration of glucose in blood and urine 818
Hirsch, Erwin O., and Gardner, Frank H. The life span of transfused human blood platelets 649
Hoch, Frederic L., and Vallee, Bert L. Characterization of leucocyte zinc protein 650
Holland, B. C. Effect of Pitressin induced water retention on sodium excretion 650
Hollander, Joseph L., Brown, Ernest M., Jr., Jessar, Ralph A., and Brown, Charles Y. Comparative effects of compound F (17-hydroxycorticosterone) and cortisone injected locally into the rheumatoid arthritic joint 650
—, **Stoner, Emery K., Brown, Ernest M., Jr., and deMoor, Peter.** Joint temperature measurement in the evaluation of anti-arthritic agents 701
Hollander, Vincent P. See PEARSON, ELIEL, and HOLLANDER 665
Hollingsworth, J. William. See HARRINGTON, HOLLINGSWORTH, MINNICH, and MOORE 646
Holze, Elizabeth A. See LEE and HOLZE 539
Homburger, F. See FISHMAN, SMITH, THOMPSON, BONNER, KASDON, and HOMBURGER 685
Hoobler, S. W., Corley, R. W., and Kabza, T. G. Effects of protoveratrine on the circulation in hypertension 651
Hopke, Charles J., Jr. See HAVENS, SHAFFER, and HOPKE 647
Hopper, James, Jr. See BROWN, HOPPER, SAMPSON, and MUDRICK 1441
Horrigan, Daniel, Jarrold, Thomas, and Vilter, Richard W. Direct action of vitamin B_{12} upon human bone marrow; the effect of instillations of vitamin B_{12} and folic acid into the bone marrow as studied by nucleic acid staining techniques 31
Horsfall, F. L., Jr. See GINSBERG and HORSFALL 640
Horwitz, Orville. See SAŸEN, SHELDON, ZINSSER, KUO, HORWITZ, and MCCALLIE 670
—. See SAŸEN, SHELDON, HORWITZ, KUO, PEIRCE, ZINSSER, and MEAD 932
Houser, Harold B. See HAHN, HOUSER, RAMMELKAMP, DENNY, and WANNAMAKER 274
Howland, Joe W., Furth, F. W., and Coulter, M. The role of infection in the radiation syndrome 651
Hubbard, William N., Jr. The systemic toxic responses of patients to treatment with streptokinase streptodornase (SK-SD) 1171
Huff, Rex L., Elmlinger, Paul J., Garcia, Joseph F., Oda, John, and Cockrell, Marion. Ferrokinetics in normal subjects and in patients having various hematopoietic disorders 651

—, **Elmlinger, Paul J., Garcia, Joseph F., Oda, John M., Cockrell, Marion C.,** and **Lawrence, John H.** Ferrokinetics in normal persons and in patients having various erythropoietic disorders 1512
Humphrey, M. J. See BARKER, HUMPHREY, and SOLEY 55
Hussey, Clara V. See QUICK and HUSSEY 903
Hyde, Grace M. See PRENTICE, BERLIN, HYDE, PARSONS, LAWRENCE, and PORT 1471

I

Ingbar, Sidney H. See KASS, INGBAR, HARRIS, and LEY 653
Ingelfinger, Franz J. See CULBERTSON, WILKINS, INGELFINGER, and BRADLEY 305
—. See WILKINS, CULBERTSON, and INGELFINGER 312
—. See KRAMER, MINKEL, FRANK, and INGELFINGER 653
Itano, Harvey A. See NEEL, WELLS, and ITANO 1120
Izzo, Anthony J. See BURTON, ZAFFARONI, IZZO, and KEUTMANN 632

J

Jacobson, L. O. See BETHARD and JACOBSON 629
Jacobson, W. E., Hammarsten, J. F., and **Heller, B. I.** The effects of adrenaline upon renal function and electrolyte excretion 1503
Jacox, Ralph F. Beta glucuronidase production by beta hemolytic streptococci 652
Jager, B. V., and **Smith, E. L.** Lack of correlation between immunologic and electrophoretic estimation of gamma globulin in human serum 652
James, G. Watson, III, Purnell, Oliver J., and **Evans, Everett I.** The anemia of thermal injury. I. Studies of pigment excretion 181
—, **Purnell, Oliver J.,** and **Evans, Everett I.** The anemia of thermal injury. II. Studies of liver function 191
Janeway, C. A. See METCOFF, KELSEY, and JANEWAY 471
Janney, Clinton D. See SHEETS, JANNEY, HAMILTON, and DEGOWIN 1272
Jarrold, Thomas. See HORRIGAN, JARROLD, and VILTER 31
Jayne, H. W. See SCHEINBERG, JAYNE, RICH, and BELLE 671
Jeffers, William A., Zintel, Harold A., Hafkenschiel, Joseph H., Dohan, Francis C., and **Keffer, Nelly J.** The blood pressure of patients with hypertension after subtotal adrenalectomy: relationship to the amount of residual adrenal tissue and substitution therapy 652
—. See HAFKENSCHIEL, CRUMPTON, SHENKIN, MOYER, ZINTEL, WENDEL, and JEFFERS 793
Jenkins, Dalton. See EMERSON, ROCHE, KAHN, MOSER, and JENKINS 637
—. See FRAWLEY, ROCHE, JENKINS, and THORN 638
Jessar, Ralph A. See HOLLANDER, BROWN, JESSAR, and BROWN 650
Johnson, Ben B. See LUETSCHER, DEMING, and JOHNSON 658
—. See LUETSCHER, DEMING, and JOHNSON 1530
Johnson, Carl E. See MAN, HEINEMANN, JOHNSON, LEARY, and PETERS 137
Johnson, Robert L. See FREIS, STANTON, FINNERTY, SCHNAPER, JOHNSON, RATH, and WILKINS 435
—. See SCHNAPER, JOHNSON, TUOHY, and FREIS 786
Johnston, Barbara. See LESLIE, JOHNSTON, and RALLI 1200
Jones, Helen S. See LOCKE, TALBOT, JONES, and WORCESTER 325
Jordan, William S., Jr., Pillemer, Louis, and **Dingle, John H.** The mechanism of hemolysis in paroxysmal cold hemoglobinuria. I. The role of complement and its components in the Donath-Landsteiner reaction 11
—, **Pillemer, Louis,** and **Dingle, John H.** The mechanism of hemolysis in paroxysmal cold hemoglobinuria. II. Observations on the behavior and nature of the antibody 22
—. See HARRIS, JORDAN, PILLEMER, and DESFORGES 646
Jungck, Edwin C. See HELLER, NELSON, MADDOCK, JUNGCK, PAULSEN, and MORTIMORE 648

K

Kabza, T. G. See HOOBLER, CORLEY, and KABZA 651
Kahn, Stanley S. See EMERSON, ROCHE, KAHN, MOSER, and JENKINS 637
Kaplan, S. A. See ASSALI, KAPLAN, FOMON, DOUGLASS, and TADA 916
Kark, Robert M. See BEST, MUEHRCKE, and KARK 629
Kasdon, S. C. See FISHMAN, SMITH, THOMPSON, BONNER, KASDON, and HOMBURGER 685
Kass, Edward H., Ingbar, Sidney H., Harris, John W., and **Ley, Allyn B.** Chemical abnormalities in the erythrocytes in sickle cell anemia, their relationship to sulfhydryl metabolism and the effects of ACTH 653
Katz, L. N. See SILBER, SNIDER, GOLDBERG, KATZ, and RADNER 1046
Keating, F. Raymond, Jr. See MCCONAHEY, KEATING, and POWER 778
Keffer, Nelly J. See JEFFERS, ZINTEL, HAFKENSCHIEL, DOHAN, and KEFFER 652
Kelsey, W. M. See METCOFF, KELSEY, and JANEWAY 471
Kety, Seymour S. See HENRY, GAUER, KETY, and KRAMER 292
Keutmann, E. Henry. See BURTON, ZAFFARONI, IZZO, and KEUTMANN 632
—. See WATERHOUSE, KEUTMANN, and FENNINGER 681
Keys, Ancel. See TUCKER and KEYS 869
Kiley, John E. See WOLF, REMP, KILEY, and CURRIE 683
—. See WOLF, REMP, KILEY, and CURRIE 1062
King, Benton D. See PRICE, KING, ELDER, LIBIEN, and DRIPPS 1243
Kingsley-Pillers, E. M. See BURCHENAL and KINGSLEY-PILLERS 631
Kinsell, Laurance W., Margen, Sheldon, Michaels, George D., and **McCallie, David P.** Studies in fat metabolism. II. Evaluation of the effect of testosterone propionate upon ketone, carbohydrate, and protein metabolism in a patient with diabetes mellitus complicated by thyrotoxicosis 1486
—, **Margen, Sheldon, Michaels, George D., Reiss, Robert, Frantz, Robert,** and **Carbone, John.** Studies in fat metabolism. III. The effect of ACTH, of cortisone, and of other steroid compounds upon fasting-induced hyperketonemia and ketonuria 1491
—. See MICHAELS, MARGEN, LIEBERT, and KINSELL 1483

Kirby, William M. M. See BURNELL and KIRBY 697
Kirsner, Joseph B. See MOELLER, RIDER, and KIRSNER 662
—. See RICKETTS, EICHELBERGER, and KIRSNER 1157
Kleeman, C. R. See LAHEY, ARST, SILVER, KLEEMAN, and KUNKEL 654
Klein, Margaret. See NATELSON, KLEIN, and KRAMER 50
Klein, Robert. Adrenocortical control of sodium and potassium excretion in the newborn period 318
Knight, Vernon. See WERNER, KNIGHT, and MCDERMOTT 682
Kory, Ross C., and **Meneely, George R.** Cardiac output in auricular fibrillation with observations on the effects of conversion to normal sinus rhythm 653
Kramer, Benjamin. See NATELSON, KLEIN, and KRAMER 50
Kramer, Kurt. See HENRY, GAUER, KETY, and KRAMER 292
Kramer, Philip, Minkel, Herbert, Frank, Heddy, and **Ingelfinger, Franz J.** Normal and abnormal swallowing mechanisms studied by the measurement of intraluminal esophagus pressures 653
Krevans, Julius R. See HARTMANN, CONLEY, and KREVANS 647
—. See HARTMANN, CONLEY, and KREVANS 948
Krivit, W. See TYLER, POLGLASE, and KRIVIT 679
Kroop, Irving G. See SIROTA and KROOP 1082
Kunkel, H. G., Ahrens, E. H., Jr., Eisenmenger, W. J., Bongiovanni, A. M., and **Slater, R. J.** Extreme hypergammaglobulinemia in young women with liver disease of unknown etiology 654
Kunkel, P. See LAHEY, ARST, SILVER, KLEEMAN, and KUNKEL 654
Kuo, Peter T. See CRUMPTON, FRIEDLAND, KUO, SCHNABEL, FOLTZ, PAGE, ALANIS, and HAFKENSCHIEL 634
—. See SAŸEN, SHELDON, ZINSSER, KUO, HORWITZ, and MCCALLIE 670
—. See SAŸEN, SHELDON, HORWITZ, KUO, PEIRCE ZINSSER, and MEAD 932
Kydd, David M., and **Man, Evelyn B.** Precipitable iodine of serum (SPI) in disorders of the liver 874
—. See ENGSTROM, KYDD, PETERS, and MAN 151
—. See MAN, KYDD, and PETERS 531

L

Lahey, W. J., Arst, D. B., Silver, M., Kleeman, C. R., and **Kunkel, P.** Physiological observations on a case of beriberi "heart disease" with a note on the acute effects of thiamine 654
Landwehr, Greta. See ALEXANDER, GOLDSTEIN, and LANDWEHR 252
—. See ALEXANDER, GOLDSTEIN, LANDWEHR, and COOK 596
—. See ALEXANDER, GOLDSTEIN, and LANDWEHR 627
Larson, Daniel L. Bacterial capsular polysaccharides in the cerebrospinal fluid of patients with purulent meningitis 582
—, and **Tomlinson, Lois J.** Quantitative antibody studies in man. I. The effect of adrenal insufficiency and of cortisone on the level of circulating antibodies 1451
Laurent, Angela M. See SALK, BAILEY, and LAURENT 669
Lawrason, F. Douglas. See EPSTEIN, GOODYER, LAWRASON, and RELMAN 63
Lawrence, John H. See BERLIN, TOLBERT, and LAWRENCE 73
—. See PRENTICE, BERLIN, HYDE, PARSONS, LAWRENCE, and PORT 1471
—. See HUFF, ELMLINGER, GARCIA, ODA, COCKRELL, and LAWRENCE 1512
Leaf, Alexander, and **Mamby, Audley R.** An antidiuretic mechanism not regulated by extracellular fluid tonicity 654
—. See BARTTER, ALBRIGHT, FORBES, LEAF, DEMPSEY, and CARROLL 237
—. See MILLER, LEAF, MAMBY, and MILLER 661
Leake, T. B. See BENTINCK, GORDAN, ADAMS, ARNSTEIN, and LEAKE 200
Leary, Deborah C. See MAN, HEINEMANN, JOHNSON, LEARY, and PETERS 137
Lee, Richard E., and **Holze, Elizabeth A.** Peripheral vascular hemodynamics in the bulbar conjunctiva of subjects with hypertensive vascular disease 539
—. Some hemodynamic aspects of the peripheral vessels in hypertensive vascular disease 654
Lehman, R. A. See GROSSMAN, WESTON, LEHMAN, HALPERIN, ULLMANN, and LEITER 1208
—. See WESTON, GROSSMAN, LEHMAN, ULLMANN, HALPERIN, and LEITER 1221
Leifer, E. See NICKEL, LOWRANCE, and LEIFER 664
Leiter, Louis. See GROSSMAN, WESTON, LEHMAN, HALPERIN, ULLMANN, and LEITER 1208
—. See WESTON, GROSSMAN, LEHMAN, ULLMANN, HALPERIN, and LEITER 1221
—. SEE WESTON, GROSSMAN, and LEITER 1262
—. See WHITE, RUBIN, and LEITER 1287
LeMaistre, Charles A., Tompsett, Ralph, Muschenheim, Carl, Moore, James A., and **McDermott, Walsh.** Effects of adrenocorticotropic hormone and cortisone in patients with tuberculosis 445
Lemon, Henry M., Looney, Joseph M., and **Chasen, William C.** Glycine metabolism in rheumatoid arthritis 655
Lennon, Beatrice. See DOBYNS and LENNON 636
Leslie, Stephen H., Johnston, Barbara, and **Ralli, Elaine P.** Renal function as a factor in fluid retention in patients with cirrhosis of the liver 1200
Lever, Walter F., Gurd, Frank R. N., Uroma, Eero, Brown, Ray K., Barnes, Benjamin A., Schmid, Karl, and **Schultz, Ethel L.** Chemical, clinical, and immunological studies on the products of human plasma fractionation. XL. Quantitative separation and determination of the protein components of small amounts of normal human plasma 99
Levine, Bennett. See WEISBERGER, HEINLE, and LEVINE 681
Levitt, Marvin F., and **Bader, Mortimer E.** Effect of cortisone and ACTH on body water and electrolyte distribution 655
Levy, Betty. See STOLLERMAN, BRODIE, LEVY, and POROSOWSKA 677
Lewis, Benjamin M., Haynes, Florence W., and **Spiegl, Ralph J.** The relation of mitral valve area to clinical status in patients with mitral stenosis 656
Ley, Allyn B., and **Gardner, Frank H.** The effect of cortisone acetate on acquired hemolytic anemia 656
—. See KASS, INGBAR, HARRIS, and LEY 653
Ley, Herbert L., Jr., and **Smadel, Joseph E.** Immunization against scrub typhus by combining living vaccine with chemoprophylaxis 656

Libien, Benjamin H. See PRICE, KING, ELDER, LIBIEN, and DRIPPS 1243
Lieberman, Seymour. See TAGNON, SCHULMAN, BRUNSCHWIG, and LIEBERMAN 678
Liebert, Gerald. See MICHAELS, MARGEN, LIEBERT, and KINSELL 1483
Lilienthal, Joseph L., Jr. See ZIERLER, ANDRES, and LILIENTHAL 684
Linenthal, Arthur J. Quantitative studies in man of the cardiac effects of reflex vagal stimulation 656
Lippman, R. W. See ADDIS, BARRETT, POO, UREEN, and LIPPMAN 206
Litter, Julius, and **Wood, J. Edwin, III.** The effect of graded external pressures on the vascular volume of the leg 657
Lloyd, C. W., Harris, J. F., and **Lobotsky, J.** Some studies of posterior pituitary and adrenal cortical interrelationships 657
Lobotsky, J. See LLOYD, HARRIS, and LOBOTSKY 657
Locke, William, Talbot, Nathan B., Jones, Helen S., and **Worcester, Jane.** Studies on the combined use of measurements of sweat electrolyte composition and rate of sweating as an index of adrenal cortical activity 325
Lodge, Anne L. See TAGER and LODGE 677
Loeb, Lenore H. See GLASER, BERRY, LOEB, and WOOD 640
Logan, M. A. See ALTEMEIER, COITH, CULBERTSON, LOGAN, and TYTELL 627
London, Irving M., Sabella, Gloria F., and **Yamasaki, Martha M.** Studies on the metabolism of cholesterol in normal man and in the nephrotic syndrome 657
Looney, Joseph M. See LEMON, LOONEY, and CHASEN 655
Lorber, Stanley H., Shay, Harry, Oppenheimer, M. J., and **Siplet, Herman.** Entero-hepatic circulation of bromsulphalein: studies in man and dog 658
Lown, Bernard, and **Ganong, Frank.** The syndrome of short P-R interval, normal QRS complex and paroxysmal rapid heart action 658
Lowrance, P. See NICKEL, LOWRANCE, and LEIFER 664
Lozner, Eugene L. See FALOON, GREENE, and LOZNER 638
Ludemann, Henry. See CRAWFORD and LUDEMANN 1456
Luetscher, John A., Jr., Deming, Quentin B., and **Johnson, Ben B.** A comparison of the effects of ACTH and cortisone in nephrosis 658
—, **Deming, Quentin B.,** and **Johnson, Ben B.** Treatment of nephrosis with pituitary adrenocorticotrophin 1530

M

MacDowell, Muriel. See OLIVER, MACDOWELL, and TRACY 1307
MacIntyre, W. J. See PRITCHARD, MACINTYRE, ECKSTEIN, and FRIEDELL 666
Mack, Harold C., Robinson, Abner R., Wiseman, Margaret E., Schoeb, Ernest J., and **Macy, Icie G.** Plasma proteins in toxemias of pregnancy 609
Macpherson, A. I. See BRADLEY, MACPHERSON, GAMMELTOFT, and BLAKEMORE 630
Macy, Icie G. See MACK, ROBINSON, WISEMAN, SCHOEB, and MACY 609
Maddock, William O. See HELLER, NELSON, MADDOCK, JUNGCK, PAULSEN, and MORTIMORE 648
Magnuson, Harold J., Tauber, Henry, McLeod, Charlotte, and **Garson, Warfield.** Protein studies on treponemal immobilizing antibodies 659
Maistereena, George. See RENOLD, MAISTEREENA, and FORSHAM 667
Mamby, Audley R. See LEAF and MAMBY 654
—. See MILLER, LEAF, MAMBY, and MILLER 661
Man, Evelyn B., Heinemann, Martin, Johnson, Carl E., Leary, Deborah C., and **Peters, John P.** The precipitable iodine of serum in normal pregnancy and its relation to abortions 137
—, **Kydd, David M.,** and **Peters, John P.** Butanol-extractable iodine of serum 531
—. See ENGSTROM, KYDD, PETERS, and MAN 151
—. See PETERS, HEINEMANN, and MAN 388
—. See KYDD and MAN 874
Manion, Robert E. See HALL and MANION 1542
Manning, Elizabeth L. See CLARK, MANNING, and APPLETON 633
Mansuy, Matthew M. See PETERS, SCHWARTZ, MERMELSTEIN, NEFORES, and MANSUY 799
Margen, Sheldon. See MICHAELS, MARGEN, LIEBERT, and KINSELL 1483
—. See KINSELL, MARGEN, MICHAELS, and MCCALLIE 1486
—. See KINSELL, MARGEN, MICHAELS, REISS, FRANTZ, and CARBONE 1491
Martin, Samuel P., Bennett, Ivan L., and **Chaudhuri, S.** The effect of a series of bacteria and their products on the human leukocyte 659
Masland, Richard L. See HARRELL, MASLAND, and ROSENBLUM 645
Mateer, F. M., Erhard, Loraine H., Price, Marjorie, Weigand, F. A., Peters, J. H., Danowski, T. S., Tarail, R., and **Greenman, L.** Sodium restriction and cation exchange resin therapy in nephrotic children 1018
—. See GREENMAN, DANOWSKI, TARAIL, PETERS, WEIGAND, and MATEER 644
—. See DANOWSKI, GREENMAN, PETERS, and MATEER 979
—. See DANOWSKI, GREENMAN, MATEER, PARSONS, WEIGAND, MERMELSTEIN, and PETERS 984
—. See GREENMAN, PETERS, MATEER, WEIGAND, WILKINS, TARAIL, RHODES, and DANOWSKI 995
—. See PETERS, DANOWSKI, GREENMAN, WEIGAND, CLARKE, GARVER, MATEER, and TARAIL 1009
—. See GREENMAN, PETERS, MATEER, and DANOWSKI 1027
Mathers, James A. L. See NICKERSON, MATHERS, and SJOBERG 664
McAdams, George B. Thyroid hyperplasia disproportionate to function 659
McCallie, David P. See SAŸEN, SHELDON, ZINSSER, KUO, HORWITZ, and MCCALLIE 670
—. See KINSELL, MARGEN, MICHAELS, and MCCALLIE 1486
McCarty, Maclyn. See WOOD and MCCARTY 616
McConahey, William M., Keating, F. Raymond, Jr., and **Power, Marschelle H.** An estimation of the renal and extrarenal clearance of radioiodide in man 778
McCrory, Wallace W. See BARNETT, FORMAN, MCNAMARA, MCCRORY, RAPOPORT, MICHIE, and BARBERO 227
McDermott, Walsh. See LEMAISTRE, TOMPSETT, MUSCHENHEIM, MOORE, and MCDERMOTT 445

—. See WERNER, KNIGHT, and MCDERMOTT 682
McDonald, Roger K., Solomon, David H., and **Shock, Nathan W.** Aging as a factor in the renal hemodynamic changes induced by a standardized pyrogen 457
—, **Miller, John H.,** and **Roach, Eleanore B.** Human glomerular permeability and tubular recovery values for hemoglobin 1041
—. See MILLER and MCDONALD 1033
McGuire, Johnson. See FOWLER, WESTCOTT, SCOTT, and MCGUIRE 517
—. See WESTCOTT, FOWLER, SCOTT, HAUENSTEIN, and MCGUIRE 957
McLeod, Charlotte. See MAGNUSON, TAUBER, MCLEOD, and GARSON 659
McNamara, Helen. See BARNETT, FORMAN, MCNAMARA, MCCRORY, RAPOPORT, MICHIE, and BARBERO 227
Mead, John, Jr. See SAYEN, SHELDON, HORWITZ, KUO, PEIRCE, ZINSSER, and MEAD 932
Meilman, E. The action of protoveratrine on renal function in hypertension 659
Mellinkoff, Sherman M. See HILDRETH, MELLINKOFF, BLAIR, and HILDRETH 649
Mendlowitz, Milton, Touroff, Arthur S. W., and **Abel, Harold A.** The effect of venous and arterial occlusion and sympathetic nerve tone, on digital blood flow 94
—. The causes of constriction of the digital vascular bed 660
Meneely, George R. See KORY and MENEELY 653
Merchant, William R., Brown, Thomas McP., Robinson, Lucille B., and **Wichelhausen, Ruth H.** Observations on the effect of intravenous human serum albumin in hypersensitivity reactions and various arthritic states 660
Mermelstein, Howard. See PETERS, SCHWARTZ, MERMELSTEIN, NEFORES, and MANSUY 799
—. See DANOWSKI, GREENMAN, MATEER, PARSONS, WEIGAND, MERMELSTEIN, and PETERS 984
Merrill, John P., Goodale, Walter T., and **Swan, Roy C.** Observations on the pressor response occurring during dialysis with the artificial kidney 660
Metcalfe, James, and **Woodbury, John W.** The effects of experimental pericardial tamponade on pressures in the pulmonary circulation 661
Metcoff, Jack, Kelsey, W. M., and **Janeway, C. A.** The nephrotic syndrome in children. An interpretation of its clinical, biochemical, and renal hemodynamic features as variations of a single type of nephron disease 471
—, **Rance, C. P.,** and **Nakasone, N.** Observations on renal mechanisms for sodium and potassium excretion in ACTH-induced diuresis of nephrotic edema 661
Michaels, George D., Margen, Sheldon, Liebert, Gerald, and **Kinsell, Laurance W.** Studies in fat metabolism. I. The colorimetric determination of ketone bodies in biological fluids 1483
—. See KINSELL, MARGEN, MICHAELS, and MCCALLIE 1486
—. See KINSELL, MARGEN, MICHAELS, REISS, FRANTZ, and CARBONE 1491
Michie, Alexander J. See BARNETT, FORMAN, MCNAMARA, MCCRORY, RAPOPORT, MICHIE, and BARBERO 227
Miller, Benjamin F., Leaf, Alexander, Mamby, Audley R., and **Miller, Zelma.** Relationship of allantoin and endogenous creatinine clearances to glomerular filtration rate in man 661
Miller, John H., and **McDonald, Roger K.** The effect of hemoglobin on renal function in the human 1033
—. See MCDONALD, MILLER, and ROACH 1041
Miller, Max. See CRAIG, MILLER, DRUCKER, WOODWARD, BROFMAN, and PRITCHARD 634
Miller, Treva B. See AIKAWA, HARRELL, and MILLER 575
Miller, Zelma. See MILLER, LEAF, MAMBY, and MILLER 661
Minkel, Herbert. See KRAMER, MINKEL, FRANK, and INGELFINGER 653
Minnich, Virginia. See HARRINGTON, HOLLINGSWORTH, MINNICH, and MOORE 646
Moeller, Hugo C., Rider, J. Alfred, and **Kirsner, Joseph B.** Erythrocyte and serum cholinesterase in ulcerative colitis 662
Mogabgab, William J. See THOMAS, MOGABGAB, and GOOD 678
Mond, Ernest, and **Singer, Karl.** Effect of platelet extract on hemophilic blood 77
Moore, Carl V. See HARRINGTON, HOLLINGSWORTH, MINNICH, and MOORE 646
Moore, F. D. See EDELMAN and MOORE 637
Moore, James A. See LEMAISTRE, TOMPSETT, MUSCHENHEIM, MOORE, and MCDERMOTT 445
Moore, Lucile V. See AYALA, MOORE, and HESS 781
Mortimore, Glenn E. See HELLER, NELSON, MADDOCK, JUNGCK, PAULSEN, and MORTIMORE 648
Moser, Hugo W. See EMERSON, ROCHE, KAHN, MOSER, and JENKINS 637
Moyer, John H. See HAFKENSCHIEL, CRUMPTON, SHENKIN, MOYER, ZINTEL, WENDEL, and JEFFERS 793
Mudge, Gilbert H. Studies on *in vitro* potassium accumulation 662
Mudrick, Charles. See BROWN, HOPPER, SAMPSON, and MUDRICK 1441
Muehrcke, Robert C. See BEST, MUEHRCKE, and KARK 629
Mueller, John F. See WEIR and MUELLER 681
Muirhead, E. E. See GROLLMAN, TURNER, and MUIRHEAD 644
Murphy, Richard J. F., and **Stead, Eugene A., Jr.** Effects of exogenous and endogenous posterior pituitary antidiuretic hormone on water and electrolyte excretion 1055
Muschenheim, Carl. See LEMAISTRE, TOMPSETT, MUSCHENHEIM, MOORE, and MCDERMOTT 445
Myers, J. D., and **Taylor, W. Jape.** An estimation of portal venous pressure by occlusive catheterization of an hepatic venule 662

N

Najjar, Victor A., and **Childs, Barton.** The crystallization of "direct" and "indirect" bilirubin from human serum and their respective properties 663
Nakasone, N. See METCOFF, RANCE, and NAKASONE 661
Natelson, Samuel, Klein, Margaret, and **Kramer, Benjamin.** The effect of oral administration of calcium fructose diphosphate on the serum organic phosphate, inorganic phosphate, calcium, protein, and citric acid levels 50

Neel, James V., Wells, Ibert C., and **Itano, Harvey A.** Familial differences in the proportion of abnormal hemoglobin present in the sickle cell trait 1120
Nefores, Mercine N. See PETERS, SCHWARTZ, MERMELSTEIN, NEFORES, and MANSUY 799
Nelson, Warren O. See HELLER, NELSON, MADDOCK, JUNGCK, PAULSEN, and MORTIMORE 648
Nelson, William P., III, and **Welt, Louis G.** The effects of Pitressin on the excretion of water and electrolytes in normal subjects and patients with cirrhosis of the liver and ascites 663
—, **Rosenbaum, Jack D.,** and **Strauss, Maurice B.** Hyponatremia in hepatic cirrhosis following paracentesis 738
Nichols, George, Jr., and **Nichols, Nancy.** Changes in plasma and erythrocyte sodium, potassium, and water during recovery from diabetic coma 663
Nichols, Mary P. See DUNCAN, SOLOMON, NICHOLS, and ROSENBERG 908
Nichols, Nancy. See NICHOLS and NICHOLS 663
Nickel, J. F., Lowrance, P., and **Leifer, E.** Effect of sodium depletion and repletion on renal function and body fluids during uremia 664
Nickerson, John L., Elkin, Daniel C., and **Warren, James V.** The effect of temporary occlusion of arteriovenous fistulas on heart rate, stroke volume, and cardiac output 215
—, **Mathers, James A. L.,** and **Sjoberg, Douglas S.** Clinical evaluation of various types of ballistocardiographs 664
—. See WARREN, NICKERSON, and ELKIN 210
—. See WARREN, ELKIN, and NICKERSON 220

O

Oda, John M. See HUFF, ELMLINGER, GARCIA, ODA, and COCKRELL 651
—. See HUFF, ELMLINGER, GARCIA, ODA, COCKRELL, and LAWRENCE 1512
Oliver, Jean, MacDowell, Muriel, and **Tracy, Ann.** The pathogenesis of acute renal failure associated with traumatic and toxic injury. Renal ischemia, nephrotoxic damage and the ischemuric episode 1307
Olson, Robert E. See GOODALE, OLSON, and HACKEL 642
Oppenheimer, M. J. See LORBER, SHAY, OPPENHEIMER, and SIPLET 658
Orloff, J. See WELT and ORLOFF 751
Ory, Alan A. See CHAPMAN and ORY 632
Osher, William J. See HECHT, OSHER, and SAMUELS 647

P

Page, E. B. See HICKAM, PRYOR, PAGE, and ATWELL 503
Page, Ernest W. See GLENDENING and PAGE 1298
Page, Irvine H. See CORCORAN, DUSTAN, and PAGE 633
Page, Robert G. See CRUMPTON, FRIEDLAND, KUO, SCHNABEL, FOLTZ, PAGE, ALANIS, and HAFKENSCHIEL 634
Paige, Emil. See BAYLES, COLPOYS, FREMONT-SMITH, FERGUSON, and PAIGE 628
Parsons, Robert J. See PRENTICE, BERLIN, HYDE, PARSONS, LAWRENCE, and PORT 1471
Parsons, W. B. See DANOWSKI, GREENMAN, MATEER, PARSONS, WEIGAND, MERMELSTEIN, and PETERS 984
Patterson, John L., Jr., and **Cannon, John L.** Postural changes in the cerebral circulation, studied by continuous oximetric and pressure-recording techniques 664
—, **Heyman, Albert,** and **Duke, T. Whatley.** The cerebral circulation and metabolism in chronic pulmonary emphysema with observations on the effects of inhalation of oxygen 665
Patterson, M., Adams, C. H., and **Stevenson, C.** Comparative studies of cortisone administered orally and intramuscularly to patients with rheumatoid arthritis 665
Paul, William D. See FRANKLIN, BEAN, PAUL, ROUTH, DE LA HUERGA, and POPPER 718
—. See FRANKLIN, BEAN, PAUL, ROUTH, DE LA HUERGA, and POPPER 729
Paulsen, C. Alvin. See HELLER, NELSON, MADDOCK, JUNGCK, PAULSEN, and MORTIMORE 648
Payne, R. W., Rosenberg, I. N., Raben, M. S., Cleroux, A. P., and **Astwood, E. B.** Effectiveness of corticotropin suspensions 665
Peacock, Wendell. See HELLMAN, PEACOCK, EIDINOFF, ROSENFELD, and GALLAGHER 648
—. See RALL, HELLMAN, PEACOCK, and RAWSON 667
Pearson, Olof H., Eliel, Leonard P., and **Hollander, Vincent P.** A comparison of the metabolic effects of the adrenal cortical steroids, Compounds A, E, F and S acetate 665
—. See ELIEL, PEARSON, and WEST 637
Peirce, George. See SAYEN, SHELDON, HORWITZ, KUO, PEIRCE, ZINSSER, and MEAD 932
Pellegrino, E. D. See EARLE, FARBER, ALEXANDER, and PELLEGRINO 421
—. See EICHNA, FARBER, BERGER, EARLE, RADER, PELLEGRINO, ALBERT, ALEXANDER, TAUBE, and YOUNGWIRTH 1250
Penneys, Raymond. An *in vivo* "oxygen dissociation curve" 666
Perera, George A. Altered metabolic response prior to the development of hypertension in a patient with hypertensive vascular disease 666
Perkoff, G. T. See TYLER, STEPHENS, GUNN, and PERKOFF 492
Perley, Anne. See FORBES and PERLEY 558
—. See FORBES and PERLEY 566
Peters, John H., Schwartz, Robert, Mermelstein, Howard, Nefores, Mercine N., and **Mansuy, Matthew M.** Studies of serum creatine tolerance. I. Findings in normal males and in hyperthyroidism 799
—, **Danowski, T. S., Greenman, L., Weigand, F. A., Clarke, C. E., Garver, K., Mateer, F. M.,** and **Tarail, R.** Acidifying and non-acidifying carboxylic resin mixtures used alone and with ACTH or cortisone 1009
—. See GREENMAN, DANOWSKI, TARAIL, PETERS, WEIGAND, and MATEER 644
—. See DANOWSKI, GREENMAN, PETERS, and MATEER 979
—. See DANOWSKI, GREENMAN, MATEER, PARSONS, WEIGAND, MERMELSTEIN, and PETERS 984
—. See GREENMAN, PETERS, MATEER, WEIGAND, WILKINS, TARAIL, RHODES, and DANOWSKI 995
—. See MATEER, ERHARD, PRICE, WEIGAND, PETERS, DANOWSKI, TARAIL, and GREENMAN 1018
—. See GREENMAN, PETERS, MATEER, and DANOWSKI 1027

Peters, John P., Heinemann, Martin, and **Man, Evelyn B.** The lipids of serum in pregnancy 388
—. See MAN, HEINEMANN, JOHNSON, LEARY, and PETERS 137
—. See ENGSTROM, KYDD, PETERS, and MAN 151
—. See MAN, KYDD, and PETERS 531
Phillips, Gerald B. See GABUZDA, PHILLIPS, SCHILLING, and DAVIDSON 639
Pillemer, Louis. See JORDAN, PILLEMER, and DINGLE 11
—. See JORDAN, PILLEMER, and DINGLE 22
—. See HARRIS, JORDAN, PILLEMER, and DESFORGES 646
Polglase, W. J. See TYLER, POLGLASE, and KRIVIT 679
Pons, E. See WOODWARD, HALL, HIGHTOWER, and PONS 683
Poo, L. J. See ADDIS, BARRETT, POO, UREEN, and LIPPMAN 206
Popper, Hans. See DE LA HUERGA and POPPER 463
—. See FRANKLIN, BEAN, PAUL, ROUTH, DE LA HUERGA, and POPPER 718
—. See FRANKLIN, BEAN, PAUL, ROUTH, DE LA HUERGA, and POPPER 729
Porosowska, Yetta. See STOLLERMAN, BRODIE, LEVY, and POROSOWSKA 677
Port, Shirley. See PRENTICE, BERLIN, HYDE, PARSONS, LAWRENCE, and PORT 1471
Power, Marschelle H. See TAYLOR, COMFORT, WOLLAEGER, and POWER 678
—. See MCCONAHEY, KEATING, and POWER 778
Prentice, Theodore C., Berlin, Nathaniel I., Hyde, Grace M., Parsons, Robert J., Lawrence, John H., and **Port, Shirley.** Total red cell volume, plasma volume and sodium space in congestive heart failure 1471
Price, Henry L., King, Benton D., Elder, John D., Libien, Benjamin H., and **Dripps, Robert D.** Circulatory effects of raised airway pressure during cyclopropane anesthesia in man 1243
Price, Marjorie. See MATEER, ERHARD, PRICE, WEIGAND, PETERS, DANOWSKI, TARAIL, and GREENMAN 1018
Pritchard, W. H., MacIntrye, W. J., Eckstein, R. W., and **Friedell, H. L.** The determination of cardiac output by a continuous recording system utilizing the injection of iodinated (I^{131}) human albumin 666
—. See CRAIG, MILLER, DRUCKER, WOODWARD, BROFMAN, and PRITCHARD 634
Proger, Samuel. See SCHMIDT, FULD, CUBILES, and PROGER 671
Pryor, W. W. See HICKAM and PRYOR 401
—. See HICKAM, PRYOR, PAGE, and ATWELL 503
Purnell, Oliver J. See JAMES, PURNELL, and EVANS 181
—. See JAMES, PURNELL, and EVANS 191

Q

Quick, Armand J., and **Hussey, Clara V.** On the comparative prothrombic activity of human and dog blood 903

R

Raben, M. S. See PAYNE, ROSENBERG, RABEN, CLEROUX, and ASTWOOD 665
Rader, Bertha. See EICHNA, BERGER, RADER, and BECKER 353
—. See EICHNA, FARBER, BERGER, EARLE, RADER, PELLEGRINO, ALBERT, ALEXANDER, TAUBE, and YOUNGWIRTH 1250
Radner, D. B. See SILBER, SNIDER, GOLDBERG, KATZ, and RADNER 1046
Ragan, Charles. See GROKOEST, VAILLANCOURT, GOTTSEGEN, and RAGAN 644
Rall, J. E., Hellman, Leon, Peacock, Wendell, and **Rawson, Rulon W.** The rate of utilization of radioiron as a measure of bone marrow damage from irradiation 667
Ralli, Elaine P. See LESLIE, JOHNSTON, and RALLI 1200
Rammelkamp, Charles H., Jr. See HAHN, HOUSER, RAMMELKAMP, DENNY, and WANNAMAKER 274
Rance, C. P. See METCOFF, RANCE, and NAKASONE 661
Rand, Royden. See BISHOP, RAND, and TALBOTT 889
Randolph, Malcolm L. See SNAVELY, GOLDWATER, RANDOLPH, SPRAGUE, and UNGLAUB 674
—. See TURNER, SNAVELY, GOLDWATER, RANDOLPH, SPRAGUE, and UNGLAUB 1071
Ransohoff, William. See BRUST, RANSOHOFF, and REISER 630
Rantz, Lowell A., and **Di Caprio, Joseph M.** Antihyaluronidase response following hemolytic streptococcal infection in childhood 667
Rapoport, Milton. See BARNETT, FORMAN, MCNAMARA, MCCRORY, RAPOPORT, MICHIE, and BARBERO 227
Rapoport, S. See BRODSKY and RAPOPORT 282
Rapport, Maurice M. See GOLDENBERG and RAPPORT 641
Rath, Charles E. See FREIS, STANTON, FINNERTY, SCHNAPER, JOHNSON, RATH, and WILKINS 435
Rawson, Rulon W. See RALL, HELLMAN, PEACOCK, and RAWSON 667
Redd, James B. See SOKOLOFF, EDDY, and REDD 395
Reifenstein, Robert W. See GRAY, REIFENSTEIN, SPIRO, and BENSON 643
Reiser, Morton F. See BRUST, RANSOHOFF, and REISER 630
—. See BRUST, REISER, and FERRIS 925
Reiss, Robert. See KINSELL, MARGEN, MICHAELS, REISS, FRANTZ, and CARBONE 1491
Relman, Arnold S. See EPSTEIN, GOODYER, LAWRASON, and RELMAN 63
Remp, Donald G. See WOLF, REMP, KILEY, and CURRIE 683
—. See WOLF, REMP, KILEY, and CURRIE 1062
Renold, Albert E., Maistereena, George, and **Forsham, Peter H.** Studies on intravenous ACTH administration 667
Rhodes, G. See GREENMAN, PETERS, MATEER, WEIGAND, WILKINS, TARAIL, RHODES, and DANOWSKI 995
Rich, M. See SCHEINBERG, JAYNE, RICH, and BELLE 671
Richer, Ruth. See STERN, BIRMINGHAM, CULLEN, and RICHER 84
Ricketts, William E., Eichelberger, Lillian, and **Kirsner, Joseph B.** Observations on the alterations in electrolytes and fluid balance in patients with cirrhosis of the liver with and without ascites 1157
Rider, J. Alfred. See MOELLER, RIDER, and KIRSNER 662
Rigas, Demetrios A., and **Heller, Carl G.** The amount and nature of urinary proteins in normal human subjects 853
Roach, Eleanore B. See MCDONALD, MILLER, and ROACH 1041
Robertson, James S. See DEANE, SCHREINER, and ROBERTSON 1463
Robinson, Abner R. See MACK, ROBINSON, WISEMAN, SCHOEB, and MACY 609
Robinson, Lucille B. See MERCHANT, BROWN, ROBINSON, and WICHELHAUSEN 660

Roche, Marcel. See EMERSON, ROCHE, KAHN, MOSER, and JENKINS 637
—. See FRAWLEY, ROCHE, JENKINS, and THORN 638
Roos, Albert. See BLACK and ROOS 338
Rose, Harry M. The effect of an inhibitor fraction from human sputum on the multiplication of influenza virus 668
Roseman, Ray H. See FRIEDMAN, FREED, and ROSEMAN 639
Rosenbaum, Jack D., Davis, Robert K., and **Ferguson, Bruce C.** The influence of cortisone on water diuresis in man 668
—. See NELSON, ROSENBAUM, and STRAUSS 738
—. See STRAUSS, DAVIS, ROSENBAUM, and ROSSMEISL 862
Rosenberg, Esther. See DUNCAN, SOLOMON, NICHOLS, and ROSENBERG 908
Rosenberg, Isadore N. The nature of the circulating thyroid hormone in Graves' disease 1
—. See PAYNE, ROSENBERG, RABEN, CLEROUX, and ASTWOOD 665
Rosenblum, Marvin J. See HARRELL, MASLAND, and ROSENBLUM 645
Rosenfeld, Robert. See HELLMAN, PEACOCK, EIDINOFF, ROSENFELD, and GALLAGHER 648
Ross, J. F., Crockett, E. C., Jr., and **Emerson, C. P.** The mechanism of anemia in leukemia and malignant lymphoma 668
Rossmeisl, Elsie C. See STRAUSS, DAVIS, ROSENBAUM, and ROSSMEISL 862
Routh, J. I. See FRANKLIN, BEAN, PAUL, ROUTH, DE LA HUERGA, and POPPER 718
—. See FRANKLIN, BEAN, PAUL, ROUTH, DE LA HUERGA, and POPPER 729
Rubin, George. See WHITE, RUBIN, and LEITER 1287
Rundles, R. Wayne, Cooper, G. R., and **Willett, R. W.** Relation of abnormal serum components to Bence Jones protein in multiple myeloma 669
—, **Cooper, Gerald R.,** and **Willett, Robert W.** Multiple myeloma. IV. Abnormal serum components and Bence Jones protein 1125
Ryan, J. M. See HICKAM, SIEKER, and RYAN 648

S

Sabella, Gloria F. See LONDON, SABELLA, and YAMASAKI 657
Salk, Jonas E., Bailey, Mary Lynch, and **Laurent, Angela M.** A safe immunologic adjuvant for enhancing the height and persistence of antibody response to influenza virus vaccines in man 669
Sampson, John J. See BROWN, HOPPER, SAMPSON, and MUDRICK 1441
Samson, Donald C., Swisher, Scott N., Christian, Richard M., and **Engel, George L.** Some observations on the mechanism of delirium in pernicious anemia 669
Samuels, A. J. Primary and secondary leucocyte changes following the intramuscular injection of epinephrine hydrochloride 941
—. See HECHT, OSHER, and SAMUELS 647
Sayen, J. J., Sheldon, W. F., Horwitz, Orville, Kuo, Peter T., Peirce, George, Zinsser, Harry F., and **Mead, John Jr.** Studies of coronary disease in the experimental animal. II. Polarographic determinations of local oxygen availability in the dog's left ventricle during coronary occlusion and pure oxygen breathing 932
—, **Sheldon, Warner F., Zinsser, Harry F., Kuo, P. T., Horwitz, Orville,** and **McCallie, David P.** Studies of coronary disease in the experimental animal. III. Polarographic studies of intramyocardial oxygen availability in dogs with acute coronary occlusion and narrowing, correlated with epicardial electrocardiograms 670
Schaefer, Louis E. See ADLERSBERG, DRACHMAN, SCHAEFER, and DRITCH 626
Schaeffer, Morris. Leptospiral meningitis. Investigation of a water-borne epidemic due to *L. pomona* 670
Scheinberg, P., Jayne, H. W., Rich, M., and **Belle, M. S.** The effects of intravenous papaverine and procaine on cerebral circulation and metabolism 671
—. See SCHIEVE, SCHEINBERG, and WILSON 671, 1527
Schieve, James F., Scheinberg, Peritz, and **Wilson, William P.** The effect of adrenocorticotrophic hormone (ACTH) on cerebral blood flow and metabolism 671, 1527
Schilling, Robert F. See GABUZDA, PHILLIPS, SCHILLING, and DAVIDSON 639
Schmid, Karl. See LEVER, GURD, UROMA, BROWN, BARNES, SCHMID, and SCHULTZ 99
Schmidt, Gerhard, Fuld, Maria, Cubiles, Ricardo, and **Proger, Samuel.** Comparative studies of the biochemistry of heart and skeletal muscle 671
Schnabel, Truman G. See CRUMPTON, FRIEDLAND, KUO, SCHNABEL, FOLTZ, PAGE, ALANIS, and HAFKENSCHIEL 634
Schnaper, Harold W., Johnson, Robert L., Tuohy, Edward B., and **Freis, Edward D.** The effect of hexamethonium as compared to procaine or methycaine lumbar block on the blood flow to the foot of normal subjects 786
—. See FREIS, STANTON, FINNERTY, SCHNAPER, JOHNSON, RATH, and WILKINS 435
Schnieder, Robert A. Relative viscosity and clotting time of whole blood in the healthy and in those with vascular disorders 672
Schoeb, Ernest J. See MACK, ROBINSON, WISEMAN, SCHOEB, and MACY 609
Schoenbach, Emanuel B., and **Weissman, Norman.** Studies on the sulfhydryl content of the serum proteins 672
—, **Weissman, Norman,** and **Armistead, Eleanor B.** The determination of sulfhydryl groups in serum. II. Protein alterations associated with disease 762
Schrager, Alvin. See BRANDT, CACCESE, DOCK, and SCHRAGER 971
Schreiner, George E. See DEANE, SCHREINER, and ROBERTSON 1463
Schrock, Christian E., Sheets, Raymond F., and **Bean, William B.** Observations during ACTH and cortisone administration to a patient with longstanding panhypopituitarism and rheumatoid arthritis 174
Schroeder, Henry A. Effects on hypertension of sulfhydryl and hydrazine compounds 672
Schubert, Maxwell, and **Einbinder, Julia.** Crystalline calcium chondroitin sulfate 673
Schulman, Phyllis. See TAGNON, SCHULMAN, BRUNSCHWIG, and LIEBERMAN 678
Schultz, Ethel L. See LEVER, GURD, UROMA, BROWN, BARNES, SCHMID, and SCHULTZ 99
Schwartz, Robert. See PETERS, SCHWARTZ, MERMELSTEIN, NEFORES, and MANSUY 799
Schwartz, William B., and **Wallace, William M.** Electrolyte equilibrium during mercurial diuresis 1089

Scott, Ralph C. See FOWLER, WESTCOTT, SCOTT, and MCGUIRE 517
—. See WESTCOTT, FOWLER, SCOTT, HAUENSTEIN, and MCGUIRE 957
Seldin, Donald W., Welt, Louis G., and **Cort, Joseph.** The effect of pituitary and adrenal hormones on the metabolism and excretion of potassium 673
—. See GOODYER and SELDIN 642
—. See WELT, SELDIN, and CORT 682
—. See TARAIL, SELDIN, and GOODYER 1111
Selverstone, Louis A. See CAMPBELL, SELVERSTONE, and DONOVAN 632
Selye, Hans. The role of somatotrophic hormone (STH) in the production of malignant nephrosclerosis, periarteritis nodosa and hypertensive disease 673
Shaffer, James M. See HAVENS, SHAFFER, and HOPKE 647
Shapiro, Arthur. See ALBAUM, CAYLE, and SHAPIRO 525
Shay, Harry. See LORBER, SHAY, OPPENHEIMER, and SIPLET 658
Sheets, Raymond F., Janney, Clinton D., Hamilton, Henry E., and **DeGowin, Elmer L.** Studies with in-agglutinable erythrocyte counts. III. Kinetics of erythrocyte destruction in human beings 1272
—. See SCHROCK, SHEETS, and BEAN 174
Sheldon, Warner F. See SAŸEN, SHELDON, ZINSSER, KUO, HORWITZ, and MCCALLIE 670
—. See SAŸEN, SHELDON, HORWITZ, KUO, PEIRCE, ZINSSER, and MEAD 932
Shenkin, Henry A., Cabieses, Fernando, and **van den Noordt, Gordon.** The effect of bilateral stellectomy upon the cerebral circulation of man 90
—. See HAFKENSCHIEL, CRUMPTON, SHENKIN, MOYER, ZINTEL, WENDEL, and JEFFERS 793
Shock, Nathan W. See MCDONALD, SOLOMON, and SHOCK 457
Sieker, H. O. See HICKAM, SIEKER, and RYAN 648
Silber, E. N., Snider, G. L., Goldberg, H., Katz, L. N., and **Radner, D. B.** The effect of khellin on cardio-pulmonary function in chronic pulmonary disease 1046
—. See GOLDBERG, SILBER, and GORDON 641
Siler, Vinton E. See WEISS, SILER, and BUECHLER 1507
Silver, M. See LAHEY, ARST, SILVER, KLEEMAN, and KUNKEL 654
Simson, Jerome. See ZIFF and SIMSON 684
Singer, Karl. See MOND and SINGER 77
Singer, R. B., Clark, J. K., and **Crosley, A. P., Jr.** Some effects of a single intravenous dose of hypertonic sodium bicarbonate in man 674
—. See ELKINTON, SQUIRES, and SINGER 381
Siplet, Herman. See LORBER, SHAY, OPPENHEIMER, and SIPLET 658
Sirota, Jonas H., and **Kroop, Irving G.** Evidence suggesting renal tubular excretion of potassium in man during recovery from acute renal insufficiency 1082
Sjoberg, Douglas S. See NICKERSON, MATHERS, and SJOBERG 664
Slater, R. J. See KUNKEL, AHRENS, EISENMENGER, BONGIOVANNI, and SLATER 654
Smadel, Joseph E. See LEY and SMADEL 656
Smith, E. L. See JAGER and SMITH 652
Smith, M. See FISHMAN, SMITH, THOMPSON, BONNER, KASDON, and HOMBURGER 685
Smythe, C. McC., Fitzpatrick, H. F., and **Blakemore, A. H.** Studies of portal venous oxygen in unanesthetized man 674
Snavely, J. R., Goldwater, W. H., Randolph, M. L., Sprague, C. C., and **Unglaub, W. G.** The lipid composition of ultracentrifugates of normal human serum in the post absorptive state 674
—. See TURNER, SNAVELY, GOLDWATER, RANDOLPH, SPRAGUE, and UNGLAUB 1071
Snider, G. L. See SILBER, SNIDER, GOLDBERG, KATZ, and RADNER 1046
Sokoloff, Boris, Eddy, Walter H., and **Redd, James B.** The biological activity of a flavonoid (vitamin "P") compound 395
Sokolow, Maurice. A study of failures in the use of quinidine for conversion of chronic auricular fibrillation 675
Soley, M. H. See BARKER, HUMPHREY, and SOLEY 55
Solomon, David H. See MCDONALD, SOLOMON, and SHOCK 457
—. See DUNCAN, SOLOMON, NICHOLS, and ROSENBERG 908
Spiegl, Ralph J. See LEWIS, HAYNES, and SPIEGL 656
Spiro, Howard M. See GRAY, REIFENSTEIN, SPIRO, and BENSON 643
Sprague, Charles C. See SNAVELY, GOLDWATER, RANDOLPH, SPRAGUE, and UNGLAUB 674
—. See TURNER, SNAVELY, GOLDWATER, RANDOLPH, SPRAGUE, and UNGLAUB 1071
Squires, Russell D., and **Elkinton, J. Russell.** The significance of hyponatremia in congestive heart failure 675
—. See ELKINTON, SQUIRES, and CROSLEY 369
—. See ELKINTON, SQUIRES, and SINGER 381
Stanbury, John B., Goldsmith, Richard E., and **Brownell, Gordon L.** The effect of thyrotropin on the release of hormone from the human thyroid 675
Stanton, Joseph R. See FREIS, STANTON, FINNERTY, SCHNAPER, JOHNSON, RATH, and WILKINS 435
Stare, Fredrick J. See VAN ITALLIE, GEYER, and STARE 680
Stead, Eugene A., Jr. See MURPHY and STEAD 1055
Stearns, Norman S., and **Callahan, Edmund J.** A clinical study of intravenous procaine amide (pronestyl): its value and hazards 676
Stefanini, Mario, and **Chatterjea, Jyoti B.** Rate of platelet survival in thrombocytopenia 676
Stephens, F. E. See TYLER, STEPHENS, GUNN, and PERKOFF 492
Sterling, Kenneth. The turnover rate of serum albumin in man as measured by I^{131}-tagged albumin 1228
—. Serum albumin turnover in Laennec's cirrhosis as measured by I^{131}-tagged albumin 1238
Stern, Karl, Birmingham, Marion K., Cullen, Andrea, and **Richer, Ruth.** Peptidase activity in leucocytes, erythrocytes and plasma of young adult and senile subjects 84
Stetson, Chandler A. The nature of the blood vessel damage in the Shwartzman phenomenon 676
Stevenson, C. See PATTERSON, ADAMS, and STEVENSON 665
Stollerman, Gene H., Brodie, Bernard B., Levy, Betty, and **Porosowska, Yetta.** The relationship of streptolysin S inhibitor to phospholipid levels in the serum of human beings and of rabbits 677
Stoner, Emery K. See HOLLANDER, STONER, BROWN, and DEMOOR 701
Strauss, Maurice B., Davis, Robert K., Rosenbaum, Jack D., and **Rossmeisl, Elsie C.** "Water diuresis" produced during recumbency by the intravenous infusion of isotonic saline solution 862

—. See NELSON, ROSENBAUM, and STRAUSS 738
Swan, Roy C. See MERRILL, GOODALE, and SWAN 660
Swanson, J. Norrie. The practical importance of the diurnal variations in number of the circulating eosinophils 677
Swisher, Scott N. See SAMSON, SWISHER, CHRISTIAN, and ENGEL 669

T

Tada, Y. See ASSALI, KAPLAN, FOMON, DOUGLASS, and TADA 916
Tager, Morris, and **Lodge, Anne L.** A plasma factor sensitive to variations in prothrombin conversion 677
Tagnon, H. J., Schulman, Phyllis, Brunschwig, Alexander, and **Lieberman, Seymour.** Colorimetric measurement of the *in vitro* action of human liver on alpha-estradiol 678
Talbot, Nathan B. See LOCKE, TALBOT, JONES, and WORCESTER 325
Talbott, John H. See BISHOP, GARNER, and TALBOTT 879
—. See BISHOP, RAND, and TALBOTT 889
Talmage, David W. See DAMMIN, BUKANTZ, DIXON, and TALMAGE 635
Tarail, Robert, Seldin, Donald W., and **Goodyer, Allan V. N.** Effects of injection of hypertonic glucose on metabolism of water and electrolytes in patients with edema 1111
—. See GREENMAN, DANOWSKI, TARAIL, PETERS, WEIGAND, and MATEER 644
—. See GREENMAN, PETERS, MATEER, WEIGAND, WILKINS, TARAIL, RHODES, and DANOWSKI 995
—. See PETERS, DANOWSKI, GREENMAN, WEIGAND, CLARKE, GARVER, MATEER, and TARAIL 1009
—. SEE MATEER, ERHARD, PRICE, WEIGAND, PETERS, DANOWSKI, TARAIL, and GREENMAN 1018
Taube, Harry. See EICHNA, FARBER, BERGER, EARLE, RADER, PELLEGRINO, ALBERT, ALEXANDER, TAUBE, and YOUNGWIRTH 1250
Tauber, Henry. See MAGNUSON, TAUBER, MCLEOD, and GARSON 659
Taylor, Ashton B., Comfort, Mandred W., Wollaeger, Eric E., and **Power, Marschelle H.** The effect of cortisone on nontropical sprue (idiopathic steatorrhea) 678
Taylor, W. Jape. See MYERS and TAYLOR 662
Thomas, Lewis, Mogabgab, William J., and **Good, Robert A.** The effects of cortisone on experimental bacterial infection and on the tissue damage produced by bacterial toxins 678
Thompson, D. B. See FISHMAN, SMITH, THOMPSON, BONNER, KASDON, and HOMBURGER 685
Thorn, George W. See FRAWLEY, ROCHE, JENKINS, and THORN 638
Tolbert, B. M. See BERLIN, TOLBERT, and LAWRENCE 73
Tomlinson, Lois J. See LARSON and TOMLINSON 1451
Tompsett, Ralph. See LEMAISTRE, TOMPSETT, MUSCHENHEIM, MOORE, and MCDERMOTT 445
Touroff, Arthur S. W. See MENDLOWITZ, TOUROFF, and ABEL 94
Towery, Beverly T. Chronic renal acidosis and severe osteomalacia with symmetrical pseudofractures: the renal impairment as related to changes in mineral metabolism 679
Tracy, Ann. See OLIVER, MACDOWELL, and TRACY 1307
Travis, Burton L., and **Ferguson, John H.** Proteolytic enzymes and platelets in relation to blood coagulation 112
Tucker, Robert G., and **Keys, Ancel.** Concentration of serum protein-bound iodine in normal men 869
Tuohy, Edward B. See SCHNAPER, JOHNSON, TUOHY, and FREIS 786
Turner, Louis B. See GROLLMAN, TURNER, and MUIRHEAD 644
Turner, Roy H., Snavely, John Robert, Goldwater, William H., Randolph, Malcolm L., Sprague, Charles C., and **Unglaub, Walter G.** The study of serum proteins and lipids with the aid of the quantity ultracentrifuge. I. Procedure and principal features of the centrifugate of untreated normal serum as determined by quantitative analysis of samples from ten levels 1071
Tyler, Frank H., Stephens, F. E., Gunn, F. D., and **Perkoff, G. T.** Studies in disorders of muscle. VII. Clinical manifestations and inheritance of a type of periodic paralysis without hypopotassemia 492
—, **Polglase, W. J.,** and **Krivit, W.** Amyotonia congenita: a symptom complex 679
Tytell, A. A. See ALTEMEIER, COITH, CULBERTSON, LOGAN, and TYTELL 627

U

Ullmann, T. D. See GROSSMAN, WESTON, LEHMAN, HALPERIN, ULLMANN, and LEITER 1208
—. See WESTON, GROSSMAN, LEHMAN, ULLMANN, HALPERIN, and LEITER 1221
Unglaub, Walter G. See SNAVELY, GOLDWATER, RANDOLPH, SPRAGUE, and UNGLAUB 674
—. See TURNER, SNAVELY, GOLDWATER, RANDOLPH, SPRAGUE, and UNGLAUB 1071
Ureen, H. J. See ADDIS, BARRETT, POO, UREEN, and LIPPMAN 206
Uroma, Eero. See LEVER, GURD, UROMA, BROWN, BARNES, SCHMID, and SCHULTZ 99

V

Vaillancourt, deGuise. See GROKOEST, VAILLANCOURT, GOTTSEGEN, and RAGAN 644
Valentine, William N., and **Beck, William S.** Biochemical studies on leukocytes 679
Vallee, Bert L. See HOCH and VALLEE 650
van den Noordt, Gordon. See SHENKIN, CABIESES, and VAN DEN NOORDT 90
Van Itallie, Theodore B., Geyer, Robert P., and **Stare, Fredrick J.** The intravenous administration of emulsions of vitamin K 680
Vilter, Richard W. See HORRIGAN, JARROLD, and VILTER 31

W

Wallace, William M. See WARMING-LARSON and WALLACE 680
—. See SCHWARTZ and WALLACE 1089
Wannamaker, Lewis W. See HAHN, HOUSER, RAMMELKAMP, DENNY, and WANNAMAKER 274
Warming-Larsen, Aage, and **Wallace, William M.** Studies of the volume and composition of sweat during diuresis in patients with nephrosis 680

Warren, James V., Nickerson, John L., and **Elkin, Daniel C.** The cardiac output in patients with arteriovenous fistulas 210
—, **Elkin, Daniel C.,** and **Nickerson, John L.** The blood volume in patients with arteriovenous fistulas 220
—, **Doyle, Joseph T., Estes, E. Harvey,** and **Wilson, Joseph S.** Patterns of pulmonary hemodynamic responses in man 680
—. See NICKERSON, ELKIN, and WARREN 215
—. See DOYLE, WILSON, ESTES, and WARREN 345
Waterhouse, Christine, Keutmann, E. Henry, and **Fenninger, Leonard D.** Studies on the mechanism of edema formation in patients with low serum electrolytes 681
Weigand, F. A. See GREENMAN, DANOWSKI, TARAIL, PETERS, WEIGAND, and MATEER 644
—. See DANOWSKI, GREENMAN, MATEER, PARSONS, WEIGAND, MERMELSTEIN, and PETERS 984
—. See GREENMAN, PETERS, MATEER, WEIGAND, WILKINS, TARAIL, RHODES, and DANOWSKI 995
—. See PETERS, DANOWSKI, GREENMAN, WEIGAND, CLARKE, GARVER, MATEER, and TARAIL 1009
—. See MATEER, ERHARD, PRICE, WEIGAND, PETERS, DANOWSKI, TARAIL, and GREENMAN 1018
Weir, David R., and **Mueller, John F.** The relationship of pyridoxine deficiency to adrenal function in the production of leucocytes 681
Weisberger, Austin S., Heinle, Robert W., and **Levine, Bennett.** On the chemical structure of compounds capable of preventing leukopenia induced by nitrogen mustard 681
Weiss, H. Vinik, Siler, Vinton E., and **Buechler, Peter R.** A quantitative relationship between the water content of skin and inflammation produced by chemical irritants 1507
Weissman, Norman. See SCHOENBACH and WEISSMAN 672
—. See SCHOENBACH, WEISSMAN, and ARMISTEAD 762
Wells, Ibert C. See NEEL, WELLS, and ITANO 1120
Welt, L. G., Seldin, D. W., and **Cort, J. H.** The effects of the pituitary and adrenal hormones on the metabolism and excretion of sodium and water 682
—, and **Orloff, J.** The effects of an increase in plasma volume on the metabolism and excretion of water and electrolytes by normal subjects 751
—. See NELSON and WELT 663
—. See SELDIN, WELT, and CORT 673
Wendel, Herbert. See HAFKENSCHIEL, CRUMPTON, SHENKIN, MOYER, ZINTEL, WENDEL, and JEFFERS 793
Werner, Charles A., Knight, Vernon, and **McDermott, Walsh.** Comparison of the antityphoid activities of aureomycin and chloramphenicol on bacteria artificially localized *in vivo* 682
West, Charles D. See ELIEL, PEARSON, and WEST 637
West, Philip M. See ELLIS and WEST 547
Westcott, Richard N., Fowler, Noble O., Scott, Ralph C., Hauenstein, Virgil D., and **McGuire, Johnson.** Anoxia and human pulmonary vascular resistance 957
—. See FOWLER, WESTCOTT, SCOTT, and MCGUIRE 517
Weston, R. E., Grossman, J., Lehman, R. A., Ullmann, T. D., Halperin, J. P., and **Leiter, L.** Renal extraction and excretion of mercury in man following intravenously administered mercurial diuretics 1221
—, **Grossman, Jacob,** and **Leiter, Louis.** The effect of mercurial diuretics on ammonia and titratable acidity production in acidotic human subjects with reference to site of diuretic action 1262
—. See GROSSMAN and WESTON 645
—. See GROSSMAN, WESTON, LEHMAN, HALPERIN, ULLMANN, and LEITER 1208
White, Abraham G., Rubin, George, and **Leiter, Louis.** Studies in Edema. III. The effect of Pitressin on the renal excretion of water and electrolytes in patients with and without liver disease 1287
Whittenberger, J. L. See BOUTOURLINE-YOUNG and WHITTENBERGER 838
Wichelhausen, Ruth H. See MERCHANT, BROWN, ROBINSON, and WICHELHAUSEN 660
Wilkins, D. See GREENMAN, PETERS, MATEER, WEIGAND, WILKINS, TARAIL, RHODES, and DANOWSKI 995
Wilkins, Robert W., Culbertson, James W., and **Ingelfinger, Franz J.** The effect of splanchnic sympathectomy in hypertensive patients upon estimated hepatic blood flow in the upright as contrasted with the horizontal position 312
—. See CULBERTSON, WILKINS, INGELFINGER, and BRADLEY 305
—. See FREIS, STANTON, FINNERTY, SCHNAPER, JOHNSON, RATH, and WILKINS 435
Willett, Robert W. See RUNDLES, COOPER, and WILLETT 669
—. See RUNDLES, COOPER, and WILLETT 1125
Wilson, Joseph S., Estes, E. Harvey, Doyle, Joseph T., and **Bloom, Walter L.** The use of dextran in the treatment of shock 682
—. See DOYLE, WILSON, ESTES, and WARREN 345
—. See WARREN, DOYLE, ESTES, and WILSON 680
Wilson, William P. See SCHIEVE, SCHEINBERG, and WILSON 671, 1527
Wintrobe, M. M. See CARTWRIGHT, HAMILTON, GUBLER, FELLOWS, ASHENBRUCKER, and WINTROBE 161
Wiseman, Margaret E. See MACK, ROBINSON, WISEMAN, SCHOEB, and MACY 609
Witham, A. C., and **Fleming, J. W.** The effect of epinephrine on the pulmonary circulation in man 707
—, **Fleming, J. W.,** and **Bloom, W. L.** The effect of the intravenous administration of dextran on cardiac output and other circulatory dynamics 897
Wolf, A. V., Remp, Donald G., Kiley, John E., and **Currie, Gordon D.** Kinetics of hemodialysis 683
—, **Remp, Donald G., Kiley, John E.,** and **Currie, Gordon D.** Artificial kidney function: kinetics of hemodialysis 1062
Wolf, Stewart. See HINKLE and WOLF 649
—. See HINKLE, EDWARDS, and WOLF 809
—. See HINKLE, EDWARDS, and WOLF 818
Wolff, Harold G. See GRAHAM, GOODELL, and WOLFF 37
Wollaeger, Eric E. See TAYLOR, COMFORT, WOLLAEGER, and POWER 678
Wood, Harrison F., and **McCarty, Maclyn.** The measurement of C-reactive protein in human sera; comparison of the clinical tests on the basis of a quantitative method 616
Wood, J. Edwin, III. See LITTER and WOOD 657
Wood, W. Barry, Jr. See GLASER, BERRY, LOEB, and WOOD 640
Woodbury, John W. See METCALFE and WOODBURY 661
Woodward, Hiram. See CRAIG, MILLER, DRUCKER, WOODWARD, BROFMAN, and PRITCHARD 634
Woodward, T. E., Hall, H. E., Hightower, J. A., and **Pons, E.** Antibiotic therapy of leptospirosis with discussion of effects upon leptospiremia 683
Worcester, Jane. See LOCKE, TALBOT, JONES, and WORCESTER 325

Y

Yamasaki, Martha M. See LONDON, SABELLA, and YAMASAKI 657
Young, W. A. See BROWN, ELLIOTT, and YOUNG 130
Youngwirth, Sol. See EICHNA, FARBER, BERGER, EARLE, RADER, PELLEGRINO, ALBERT, ALEXANDER, TAUBE, and YOUNGWIRTH 1250

Z

Zaffaroni, Alejandro. See BURTON, ZAFFARONI, IZZO, and KEUTMANN 632
Zamcheck, Norman. Physiology of hemorrhagic shock in man. Bromsulphalein retention, circulating eosinophiles, and skin reactive hyperemia 683
Zierler, Kenneth L., Andres, Reubin, and **Lilienthal, Joseph L., Jr.** Transfer rates across muscle cell membranes in man: measurement of blood flow and fluid compartments in skeletal muscle 684
Ziff, Morris, and **Simson, Jerome.** Body water, body chloride and plasma volume changes in patients receiving ACTH therapy 684
Zinsser, Harry F. See SAŸEN, SHELDON, ZINSSER, KUO, HORWITZ, and MCCALLIE 670
—. See SAŸEN, SHELDON, HORWITZ, KUO, PEIRCE, ZINSSER, and MEAD 932
Zintel, Harold A. See JEFFERS, ZINTEL, HAFKENSCHIEL, DOHAN, and KEFFER 652
—. See HAFKENSCHIEL, CRUMPTON, SHENKIN, MOYER, ZINTEL, WENDEL, and JEFFERS 793

A

Abortion
Relation of precipitable iodine of serum to, 137
Absorption, Gastrointestinal
Using anastomotic portal veins, 263
Acceleration
Effect on cerebral blood flow, 292
Accumulation Gradient
Correlation with uptake of I^{131} in thyroid, 301
Acidosis, Chronic Renal
In osteomalacia, 679
Intracellular cation exchanges in, 381
Mercurial diuretics, effect on renal ammonia and titratable acidity, 1262
Addison's Disease. See also Adrenals
P-R interval in, 658
Adenine Nucleotide
Method for estimation of, 525
Adenylic Acid, 628
Adrenal Cortical Preparations
Compound F (17-hydroxy-corticosterone)
Comparison with Compounds A, E, and S acetate, 665
Local effect on rheumatoid joint, 650
Cortisone (Compound E)
Analgesic effect, 644
And ACTH in thrombocytopenia, 638
And cation exchange resins, 1009
And joint temperature, 701
Changes in connective tissue reaction induced by, 636
Comparison with Compounds A, F and S acetate, 665
In adrenogenital syndrome associated with congenital adrenal hyperplasia, 237
In brucellosis, 626
In chronic hepatic disease, 647
In hypertension, 630
In lymphosarcoma, 547
In nephrosis, 658
In non-tropical sprue, 678
In osteogenic sarcoma, 547
In panhypopituitarism and rheumatoid arthritis, 174
In rheumatic diseases, 628
In rheumatoid arthritis, 633, 665
In tuberculosis, 445
Local effect on rheumatoid joint, 650
Loss of electrolytes after, 637
On acute bacterial infection, 640
On bacterial infections and tissue damage from toxins, 678
On body water and electrolyte distribution, 655
On cholinesterase levels in ulcerative colitis, 662
On circulating antibodies, 1451
On fat metabolism, 1491
On idiopathic acquired hemolytic anemia, 656
On potassium metabolism, 673
On pyridoxine deficiency—hematologic effects, 681
On serum lipids, 626
On sodium and water metabolism, 682
On streptococcal infections and complications, 274
On uric acid pool and turnover rate, 879
On water diuresis and salt excretion, 668
Prevention of toxic effects of, 644
Desoxycorticosterone acetate
In nephrosis, 1530
In rheumatoid arthritis, 633
On mercury excretion, 1208
On potassium metabolism, 673
On sodium and potassium in newborn, 318
On water and sodium metabolism, 682
Desoxycorticosterone glucoside, effect on cerebral blood flow and metabolism, 200
Adrenal Medullary Preparations
Administration, chronic, 908
Adrenalin, effect on renal function and electrolyte excretion, 908, 1503
Epinephrine
And pulmonary circulation, 707
In urine, 641
Leukocyte changes after, 941
Relation to acute hypoferremia, 161
Nor-epinephrine
Effect on pulmonary arteriolar resistance, 517
In urine, 641
Adrenals
Adrenalectomy
Blood pressure in patients with hypertension after, 652
Effect on renal hemodynamics of subtotal, 633
Control of sodium and potassium excretion in newborn, 318
Cortex and eosinopenia, 629
Cortical activity measured by sweat composition and rate, 325
Cortical function and pyridoxine deficiency, 681
Cortical relationship to acute hypoferremia, 161
Cortical relationship to posterior pituitary, 657
Cortical reserve in hyperthyroidism, 635
Cortical secretion in malignancy, 632
Corticoids, formaldehydogenic: normal values and observations on disease states, 633
Effect on gastric secretion, 643
Hyperplasia, congenital, with adrenogenital syndrome, 237
In anoxia, 638
Insufficiency
Effect on circulating antibodies, 1451
Intravenous ACTH in, 667
Loss of electrolytes after cortisone and ACTH suggestive of, 637
Intravenous ACTH test for cortical function, 667
Role of somatotrophic hormone in certain diseases, 673
Adrenocorticotrophic Hormone. See Pituitary Preparations
Afibrinogenopenia
Labile factor in "serum," 252
Age
As a factor in renal hemodynamic changes induced by pyrogens, 457
Agglutination
Klebsiella pneumoniae with antiserum of pneumococcus, 582
Albumin. See Blood, serum, albumin
Allantoin
Relation to inulin clearance, 661
Alveolar Gas Exchange. See also Lungs
Effect of lipiodol on, 338
Amethopterin
Effect on sulfhydryl groups, 762
In leukemia, 631
Amino Acids. See also Blood, serum, proteins
β-alanine, in heart and skeletal muscle, 671
Aminophylline
Effect on mercury excretion, 1208
In edema of liver cirrhosis, 1157
Ammonium Chloride
Effect on mercurial diuresis, 1208
In edema of liver cirrhosis, 1157
Potentiation of mercurial diuretic by, 1105
Amyotonia Congenita
Causes of, 679
Analgesia
Effect of cortisone and ACTH, 644
Analgesics
Effect on visceral pain, 406
Anemia. See also Blood
Hemolytic factor in lymphatic leukemia, 139
Hypoferremia in dogs and relationship of adrenal cortex, 161
Mechanism of production in lymphoma and leukemia, 668, 1272
Of burns, 181, 191
Pulmonary hemodynamics in, 680

Anesthesia, Spinal
Effect on digital blood flow, 94
Effect on renal function in pregnancy, 916
In evaluation of neurogenic control of hypertension, 925
Anoxia
And human pulmonary vascular resistance, 957
Role of pituitary adrenocortical system in response to, 638
Antibiotics. See also Aureomycin; Chloramphenicol; Penicillin; Streptomycin; Terramycin
Penicillin and Benemid, 697
Therapy of leptospirosis, 683
Antibody
Altered by adrenal insufficiency and cortisone, 1451
In paroxysmal cold hemoglobinuria, 22
Response to adjuvant-enhanced influenzal vaccine, 669
Response with I^{131}, 634
Antidiuresis
A mechanism not regulated by extracellular fluid tonicity, 654
Effect of antidiuretic hormone on water and electrolyte excretion, 1055
Of quiet standing; plasma volume and glomerular filtration rate changes in, 63
Antihyaluronidase Titers
Compared to antistreptolysin O, 667
Antipyrine
Determination of total body water, 1469
Antistreptolysin
In acute nephritis, 421
O titers, compared to antihyaluronidase, 667
Relation to phospholipids, 677
Appendicitis
During cortisone therapy, 274
Arthritis, Experimental
Role of somatotrophic hormone, 673
Arthritis, Rheumatoid
Effect of albumin, 660
Effect of cortisone orally and intramuscularly, 665
Effect of injection of compound F or cortisone into joint, 650, 701
Fasting-induced ketonuria and steroids in, 1491
Metabolism of glycine in, 655
Uric acid pool size and turnover rate in, 879
Arterial Occlusion
Effect on digital blood flow, 94
Arterial Oxygen Saturation
Effect of lipiodol on, 338
Arteriolar Tone
In postural hypotension, 401
Arteriovenous Fistula
Blood volume in, 220
Cardiac output in, 210
Effect of occlusion on, 215
Ascites
Effect on renal function, 1200
Ashby Counts, 1272
Asthma, Bronchial
Procaine amide in, 676
Aureomycin
Antityphoidal effect compared with chloramphenicol, 682
Therapy of leptospirosis, 683
Auricular Fibrillation, Chronic
Causes of conversion failures with quinidine, 675

B

Bacteria
Cross agglutination of *Klebsiella pneumoniae* with pneumococcus, 582
Effect of p-hydroxybenzoic acid on *Escherichia coli*, 635
Effect on leukocytes, 659
Immunization in *Clostridium novyii* gas gangrene, 627
Mycobacterium tuberculosis, effects of ACTH and cortisone on, 445
Polysaccharides, in *Klebsiella pneumoniae* meningitis, 582
S. typhosa, in vivo effect of chloramphenicol and aureomycin on, 682
Bacterial Infections
Effect of cortisone on, 640, 678
Bacterial Products
Effect of cortisone on action of, 678
Effect on leukocytes, 659
Ballistocardiograms
Comparison of longitudinal, lateral and dorsoventral, 971
Ballistocardiograph. See also Heart
Evaluation of types, 664
Basal Metabolic Rate
And serum creatine tolerance, 799
Bence-Jones Protein
Relation to abnormal serum components, 669
Benemid
Effect on uric acid metabolism, 889
Elevation of serum penicillin, 697
Beriberi
Heart failure in, 632
Beta-Alanine
Of heart and skeletal muscle, 671
Beta Glucuronidase
Production by beta hemolytic streptococci, 652
Bilirubin. See Blood, serum, bilirubin
Blood. See also Anemia; Leukemia
Anemia
Cortisone effect on idiopathic acquired hemolytic anemia, 656
Hemolytic factor in lymphatic leukemia, 130
Hemolytic, in lupus erythematosus, 636
Blocking antibodies in tuberculosis, 1542
Clotting time, relation to blood viscosity, 672
Coagulation
Ac-globulin, 596
Congenital deficiency with hemorrhage, 596
Consumption of labile factor, 252
Effect of paritol on, 632
Influence of pH on, 629
Relation of coagulase reacting factor to, 677
Relation of proteolytic enzymes and platelets to, 112
Serum prothrombin conversion accelerator, 596
Site of inhibition by trypsin inhibitor, 1298
Eosinophils
Diurnal variations in, 677
In adrenogenital syndrome with congenital adrenal hyperplasia, 237
In nephrosis with ACTH, 1530
In shock, 683
Response following intravenous ACTH, 667
Erythrocytes
Cholesterol, relation to serum cholesterol, 657
Cholinesterase in ulcerative colitis, 662
Peptidase activity in, 84
Potassium of, in treated diabetic coma, 663
Random destructive mechanism, 1272
Sensitized with tuberculin, 1542
Sodium of, in treated diabetic coma, 663
Water of, in treated diabetic coma, 663
Ferrokinetics, 651, 1512
Flow. See also various organs
Digital, effect of venous and arterial occlusion and sympathetic nerve tone, 94
Effect of hexamethonium, 786
Effect of sympathetic block, 786
In cerebrum, 671, 1527
In cirrhosis, 630
In muscle, 684
Peripheral, new method compared to plethysmography, 684
Glucose
Desoxycorticosterone glucoside, effect on, 200
Following stress in diabetes mellitus, 818
Stress, effect on, 809
Hemagglutinins, in pulmonary tuberculosis, 1542
Hematocrit, in nephrosis, 1530
Hemoglobin
Abnormal, in sickle cell trait, 1120

Measure of synthesis, with radioiron, 667
Hemolysins, in pulmonary tuberculosis, 1542
Hemolysis, mechanism in paroxysmal cold hemoglobinuria, 11, 22
Heparin, analysis of factors influencing anticoagulant activity, 647
Idiopathic thrombocytopenic purpura, thrombocytopenic factor in, 646
Ketone
Determination, 1483
Following stress in diabetes mellitus, 818
Kinetics of erythrocyte destruction, 1272
Leukocytes
After streptokinase-streptodornase, 1171
Biochemical studies on, 679
Changes after intramuscular injection of epinephrine, 941
Characterization of zinc protein of, 650
Effect of bacteria and products on, 659
Effect of pyridoxine deficiency and cortisone in mice, 681
Peptidase activity in, 84
Role in Shwartzman phenomenon, 676
Level of adenine nucleotides, 525
Life span of transfused human blood platelets, 649
Lipids, separation of mixtures, 626
Oxygen
Content of arterial blood, 503
Content of portal venous blood, 674
Dissociation curve, *in vivo*, 666
Saturation, during acceleration, 292
Paroxysmal nocturnal hemoglobinuria; enzymatic nature of a factor in hemolysis, 646
Pernicious anemia, hemolytic factor in, 1272
Plasma
Ac-globulin. See Blood, coagulation
Albumin. See Blood, serum, albumin
Coagulase-reacting factor; reduction following plasma conversion to serum, 677
Electrophoretic studies in liver disease, 718, 729
Factor V. See Blood, plasma, labile factor
Fractionation; quantitative separation and determination of protein components, 99
Labile factor, consumption in various conditions, 252
Peptidase activity in, 84
Potassium of, in treated diabetic coma, 663
Proteins in toxemias of pregnancy, 609
Proteins, treponemal immobilizing antibodies of, 659
Prothrombin conversion, labile factor consumption in various conditions, 252
Sodium of, in treated diabetic coma, 663
Substitutes, dextran in shock, 682
Volume changes after ACTH, 684
Volume changes with quiet standing, 63
Volume, effect of increase on excretion of water and electrolytes, 751
Volume in congestive failure, 1471
Volume in nephrotic syndrome, 227
Volume of forearm, 684
Water of, in treated diabetic coma, 663
Platelets
Effect on hemophilic blood, 77
In relation to blood coagulation, 112
Life span of, 649
Rate of destruction in thrombocytopenia, 676
Role in Shwartzman phenomenon, 676
Pressure
Effect of raised airway pressure during anesthesia, 1243
Hypotensive agents, 435
Peripheral vascular hemodynamics in the bulbar conjunctiva, 539
Pressure, high
ACTH and cortisone during autonomic blockade in, 630
Altered metabolic response prior to development of, 666
Clotting time and viscosity of blood in, 672
Dietary treatment of, 584
Effect of subtotal adrenalectomy in, 633, 652
Epinephrine in urine in, 641
Evaluation of renal resistance in, 1143
Hemodynamics of peripheral vessels in, 654
Hydrazine compounds in, 672
Neurogenic control in, 925
Potassium deprivation in, 639
Protoveratrine on circulation in, 634, 651, 659
Role of somatotrophic hormone in, 673
Sulfhydryl compounds in, 672
Sympathectomy, effect on hepatic flow in various positions in, 312
Therapy with sodium depletion, 584
Tilting, effect on cerebral circulation in, 793
Upright posture, effect on hepatic blood flow in, 305
Vasconstriction in, 660
Pressure, low
Cardiac output in postural hypotension, 401
Cause in pregnant women during high spinal anesthesia, 916
Following procaine amide intravenously, 676
Postural, cerebral blood flow in, 664
Treatment with artificial kidney, 660
Protein, determination in small amounts of human plasma, 99
Proteolytic enzymes, in relation to blood coagulation, 112
Prothrombin activity of human and dog blood, 903
Red cell volume in congestive failure, 1471
Reservoirs in man, 1441
Serum
Abnormal components, and Bence-Jones protein, 1125
Albumin, in hypersensitivity reactions and various arthritic states, 660
Albumin, salt poor, in cirrhosis of liver, 1157
Albumin, tagged with radioiodine for cardiac output, 666
Albumin turnover in liver cirrhosis, 1238
Albumin turnover rate, 1228
Bilirubin, crystallization and properties, 663
Butanol-extractable iodine, 531
Calcium, correction of bleeding with fractions of, 596
Calcium, effect of oral calcium fructose diphosphate, 50
Calcium, in plasma, 631
Carbon dioxide, high, use of artificial respiration, 838
Cholesterol metabolism in normals and in nephrotic syndrome, 657
Cholesterol, position in quantity ultracentrifugation, 674, 1071
Cholesterol, relation to protein-bound iodine, 869
Cholinesterase, in ulcerative colitis, 662
Citric acid levels, effect of calcium fructose diphosphate, 50
C-reactive protein measurement, 616
Creatine tolerance, in normals and hyperthyroidism, 799
Electrolytes, during mercurial diuresis, 1089

Electrolytes, low, and edema, 681
Electrophoretic patterns in Bence-Jones proteinemia, 1125
Electrophoretic patterns in sickle cell trait, 1120
Electrophoretic studies in liver disease, 718, 729
Enzyme inhibitors in cancer therapy, 547
Estimation of gamma globulin by immunologic and electrophoretic methods, 652
Gamma globulin, in tuberculosis, 445
Hyperglobulinemia in young women with liver disease, 654
Inorganic phosphate, effect of oral calcium fructose diphosphate, 50
Labile factor consumption in various conditions, 252
Lipids in pregnancy, 388
Lipids, ultracentrifugal distribution, 674, 1071
Lipoproteins, relation to streptolysin S inhibitor, 677
Neutral fat, position in ultracentrifugation, 674, 1071
Organic phosphate, effect of oral calcium fructose diphosphate, 50
Phospholipids, position in quantity ultracentrifugation, 674, 1071
Phospholipids, relation to streptolysin S inhibitor, 677
Potassium, low, effect on hypertension, 639
Precipitable iodine. See also Serum, protein-bound iodine
Precipitable iodine and serum creatine tolerance, 799
Precipitable iodine in liver disease, 874
Precipitable iodine in pregnancy, 137, 151, 388
Protein-bound iodine. See also Blood, serum, precipitable iodine
Protein-bound iodine, clinical determination of, 55
Protein-bound iodine, normal values, 55, 869
Protein-bound iodine, radioactive, determinations of, 1
Proteins, abnormal, in multiple myeloma, 669
Proteins, sulfhydryl content of, in disease, 672
Proteins, treponemal immobilizing antibodies of, 659
Proteins, ultracentrifugal distribution of, 1071
Prothrombin and trypsin inhibitor, 1298
Sodium, low, and edema, 681
Sodium, low, in cirrhosis after paracentesis, 738
Sodium, low, significance in congestive heart failure, 675
Sulfhydryl groups associated with disease, 762
Sickle cell anemia, relation of sulfhydryl metabolism and effects of ACTH, 652
Survival of transfused erythrocytes, interpretation of curves, 1272
Thrombocytopenia, studies with ACTH and cortisone, 638
Thromboplastin, infusions of, on heparin tolerance, 948
Transfusion, 1272
Trapped in congested limbs, 435
Venous congestion, 435
Vessels
Damage in Shwartzman phenomenon, 676
In bulbar conjunctiva, 539
Pulmonary arteriolar resistance, 517
Viscosity, relation to clotting time, 672
Blood, Volume
And pulmonary arterial pressure, 345
Determination
With carbon monoxide, 1441
With radioiron, 1512
With T-1824, 435, 1441
In arteriovenous fistula, 220
In serum sickness, 360
In trichinosis, 575
Of congested limbs, 1441
Relationship to venous pressure, 1471
Body
Chloride, changes after ACTH, 684
Surface counting rates for radioiron, 1512
Water
Changes after ACTH, 684
Effect of cortisone and ACTH on, 655
Effect of sodium depletion and repletion on, 664
Studies with deuterium oxide, 637
Bone Marrow
Action of vitamin B_{12} and folic acid upon, 31
Measure of damage to, with radioiron, 667
Brain
Blood flow
Effect of ACTH on, 671, 1527
Effect of bilateral stellectomy on, 90
Effect of desoxycorticosterone glucoside on, 200
Effect of postural changes on, 664
Effect of tilting on, in hypertension, 793
Following papaverine and procaine, 671
In emphysema, 665
Maintenance factors during gravitational stress, 292
Metabolism
Effect of ACTH on, 671, 1527
Effect of desoxycorticosterone glucoside on, 200
In emphysema, 665
Bromsulphalein
Entero-hepatic circulation of, 658
Retention, in shock, 683
Bronchospirometry, 639
Brucellosis
Cortisone in, 626
Burns
Anemia of, 181
Liver function in, 191
Pathogenesis of acute renal failure following, 1307

C

Calcium. See Blood, serum, calcium
Calcium Fructose Diphosphate
Effect on serum organic and inorganic phosphate, calcium, protein and citric acid levels, 50
Cancer. See also organs; Carcinoma; Tumor
Effect of chemotherapy on sensitivity of serum enzyme inhibitors in, 547
Carbon Tetrachloride
Pathogenesis of acute renal failure following, 1307, 1317, 1338
Carboxylic Cation Exchange Resins
Acidifying and non-acidifying, with and without ACTH and cortisone, 1009
Biochemical changes of exchanger in hydrogen, ammonium, sodium, potassium or calcium form, 995
Clinical utility, 1027
Introduction to studies, 979
Studies in dogs, 984
Therapy in nephrotic children, 1018
Carcinoma. See also Cancer
Of prostate, effect of progesterone, 547
Sulfhydryl content of serum proteins, 672
Cardiac Arrhythmias
Use of procaine amide, 676
Cardiac Function
Effect of khellin, 1046
Cardiac Output. See Heart, output
Cartilage
Fractionation of chondroitin sulfate, 673
Cerebral. See also Brain
Vascular resistance and ACTH, 1527
Cerebrospinal Fluid
Polysaccharides in meningitis, 582
Children
Adrenocortical control of sodium and potassium, 318

Chloramphenicol
Antityphoidal effect compared with aureomycin, 682
Treatment of leptospirosis, 683
Chloride Excretion
During mercurial diuresis, 1089
In mercurial and ammonium chloride diuresis, 1105
Cholesterol
Diets to reduce blood levels of, 649
Choline
Urinary excretion of its metabolites, 463
Chondroitin Sulfate
Separation of crystalline and amorphous forms, 673
Cirrhosis. See Liver
Citrovorum Factor
In leukemia, 631
Clinical Investigation
Inquiry into meaning of, 623
Clotting. See Blood, clotting and coagulation
Coarctation of Aorta
Vasoconstriction in, 660
Colchicine
Effect on uric acid pool and turnover rate, 879
Collapse
Produced by venous congestion of the extremities or by venesection following certain hypotensive agents, 435
Complement
Role in Donath-Landsteiner reaction, 11
Congestive Heart Failure. See Heart, failure
Connective Tissue
Effect of cortisone on, 636
Coomb's Test
In leukemia and lymphoma, 668
Coronary Occlusion and Narrowing. See also Heart
Oxygen therapy and electrocardiograms in dogs, 670
Corticoids. See Adrenals
Cortisone. See Adrenal Cortical Preparations
Cozymase, 628
Creatinine Clearance
Following cortisone, 668
Creatinine Clearance, Endogenous
Effect of protein consumption on, 206
Relation to inulin clearance, 661
Crushing Injury
Pathogenesis of acute renal failure following, 1307
Cushing's Syndrome. See also Pituitary
P-R interval in, 658
Cyclopropane
Circulatory effects of raised airway pressure during anesthesia, 1243
Cysteine
In prevention of leukopenia following nitrogen mustard, 681
Cystinuria
Sulfhydryl content of serum proteins in, 672

D

Delirium
Mechanism in pernicious anemia, 669
Desoxycorticosterone Acetate. See Adrenal Cortical Preparations
Detergent
Effect on streptolysin S inhibitor and phospholipids, 677
Deuterium Oxide, 637
Dextran
Effect of intravenous administration, 897
In shock, 682
Diabetes Insipidus
Mechanism of polyuria, 282
Diabetes Mellitus
Diuresis and stress in, 818
Effect on maximum capacity of renal tubules to reabsorb glucose, 125
Effect on metabolism of heart muscle, 642
Insulin resistance and fat metabolism in, 1491
Variations in blood glucose in, 649
Dialysis
With artificial kidney, pressor response following, 660
Dicumarol
Labile factor in serum, 252
Reversal of effect with intravenous vitamin K_1, 680
Diet
Therapy of hypertension, 584
To reduce human serum cholesterol, 649
Di-Ethylene Glycol
Pathogenesis of acute renal failure following, 1307, 1322, 1339
Digestion
Effect on portal venous oxygen content, 674
Digits
Causes of vasoconstriction in, 660
Digoxin
In congestive failure, 1250
Dihydroergocornine
Effect on arterial pressure, 435
Diphenylamine
Purple color reaction in rheumatic fever, 781
Diuresis. See also Kidney
ACTH, effect in nephrosis, 661, 1530
In nephrosis, study of sweat during, 680
In stressful situations, 809
Mercurial
Diuretics, site of action, 1262
Electrolyte equilibrium during, 1089
Excretion by kidney, 1221
Potentiation by ammonium chloride, 1105
Nocturnal, in heart and liver disease, 642, 1191
Of stress in diabetes mellitus, 818
With recumbency and intravenous isotonic saline, 862
Diurnal Variations
Of creatinine clearance, 206
Of eosinophil count, 677
Of water and sodium in heart and liver diseases, 642, 1191
Donath-Landsteiner Reaction
Role of complement and its components in, 11

E

Eclampsia
Pathogenesis of acute renal failure following, 1307
Edema
Mechanism with low serum electrolytes, 681
Studies in, 1287
Treatment with hypertonic glucose solutions, 1111
Eisenmenger's Complex, 641
Electrocardiogram. See also Heart
Effect of oxygen following coronary occlusion in dogs, 670
Electroencephalogram
In pernicious anemia, 669
Electrolytes
And carboxylic cation exchange resins, 979, 984, 995, 1009, 1018, 1027
Diurnal variations in excretion in heart and liver disease, 642, 1191
During mercurial diuresis, 1089
Effect of cortisone and ACTH on, 665
Effect of exogenous and endogenous antidiuretic hormone on, 1055
Effect of Pitressin on, 663
Excretion
Effect of adrenalin on, 908, 1503
Effect of chronic administration of medullary hormones on, 908
Following cortisone, 668
In liver disease with Pitressin, 1287
In pregnancy with high spinal anesthesia, 916
With increased plasma volume, 751
Following hypertonic glucose in edematous patients, 1111
In ACTH-treated nephrosis, 661
In cirrhosis of liver, 1157
Intracellular cation exchanges
In acidosis, 381
In alkalosis, 369
Intravenous digoxin effects in congestive failure, 1250

Loss from body after cortisone and ACTH, 637
Metabolism in acute glomerulonephritis, 421
Potassium accumulation in tissue slices, 662
Renal ammonia and titratable acidity production in acidosis, 1262
Sodium space in congestive failure, 1471

Electrophoresis
Comparison with immunologic method of estimating gamma globulin, 652

Electrophoretic Patterns
Effect of intravenous albumin, 660

Emphysema. See Lungs, emphysema

Enzymes
Proteolytic, relation to blood coagulation, 112

Eosinopenia
Due to adrenal cortex, 629

Eosinophils. See Blood, eosinophils

Epinephrine. See Adrenal Medullary Preparations

Esophagus
Studies of intraluminal pressures in swallowing, 653

Estrogens
Inactivation by liver slices, 678

Exercise
Respiratory regulation during, in unconditioned subjects, 503

Extracellular Fluid. See also Electrolytes
Measurement by sucrose, 1463

Extracellular Volume
Effect of increase on water excretion, 862

Extremity, Lower
Effect of pressure on vascular volume of, 657

Eye
Peripheral vascular hemodynamics in the bulbar conjunctiva, 539
Thyroid tumor transplanted to anterior chamber of, 636

F

Fat Metabolism, 1483, 1486, 1491

Ferrokinetics, 651, 1512

Fibrinolysin
Relation to blood coagulation, 112

Fick Principle
Use without gas analysis, 645

Flavonoid Compound
Biological activity, 395

Folic Acid
Effect of instillations into bone marrow, 31
X-methyl in leukemia, 631

Food
Effect on eosinophil test, 677

G

Genetic Mechanisms
In familial periodic paralysis, 492
In sickle cell trait, 1120

Glomerular Filtration Rate. See also Kidney, function
Effect of protoveratrine on, 659
Relation of allantoin and creatinine to, 661

Glomerular Permeability
Effect of hemoglobin on, 1041

Glomerulonephritis
Effect of ACTH and cortisone in, 658
Sulfhydryl content of serum proteins in, 672

Glucose
Hypertonic, and edema, 1111
Reabsorption by kidney, effect of insulin and diabetes mellitus, 125
Utilization by brain, following ACTH, 671

Glucuronic Acid
In cancer of the breast, 685
Metabolism of, 685
Method for determination, 685
Tolerance curves in human beings, 685

Glycine
Radioactive, metabolism in man, 73

Glycogen
In amyotonia congenita, 679

Gonads. See also Testis
Testosterone propionate and fat metabolism, 1486, 1491

Gout
Effect of albumin in, 660
Effect of Benemid in, 889
Uric acid pool size and turnover rate in, 879

Gravity
Effect of increase on cerebral circulation, 292

Guanazolo
In acute leukemia, 547

H

Heart
Arrhythmias, use of procaine amide, 676
Beriberi heart disease, acute effects of thiamine on, 654
Blood volume in congestive failure, 1471
Circulation, effect of increased airway pressure during anesthesia, 1243
Disease
Clotting time and viscosity of blood in, 672
Use of quinidine in, 675
Dynamics of Eisenmenger's complex, 641
Failure
High output in beriberi, 632
Nocturnal diuresis of water and sodium in, 642, 1191
Pulmonary hemodynamics in, 680
Significance of hyponatremia in, 675
Function, effect of khellin on, 1046
Hemodynamic effects of conversion of atrial fibrillation to normal sinus rhythm, 647
Hypoxia as a respiratory stimulant in cardiac dyspnea, 648
Intravenous digoxin in congestive failure, 1250
Metabolism and initial fiber length, 630
Mitral stenosis
Effect of valvuloplasty in, 643
Pulmonary hemodynamics in, 680
Relation of valve area to clinical status in, 656
Motion of the thorax during cardiac cycle, 971
Muscle
Biochemistry of, 671
Effect of mild diabetes and insulin on, 642
Output, 517
By Fick principle without gas analysis, 645
By radioactive albumin, 666
By T-1824, 897
Effect of dextran on, 897
Effect of occlusion of arteriovenous fistula on, 215
Following dialysis, 660
In arteriovenous fistulas, 210
In atrial fibrillation and normal sinus rhythm, 653
In infarction, 640
In postural hypotension, 401
Physiology, effects of reflex vagal stimulation, 656
Rate, in arteriovenous fistula, 215
Stroke volume, in arteriovenous fistula, 215
Studies of coronary disease with polarograph, 932

Hemodialysis
Kinetics in artificial kidney, 683, 1062

Hemoglobin. See also Blood
Effect on renal function, 1033
Renal excretion of, 1041

Hemoglobinuria, Paroxysmal, Cold
Pathogenesis of acute renal failure following, 1307, 1326

Hemolysis
In burns, 181

Hemolytic Factor
In anemia of lymphatic leukemia, 130, 1272

Hemophilia
Effect of platelet extract on blood of, 77
Labile factor in serum of, 252

Hepatic Blood Flow. See Liver, blood flow
Hepatitis
C-reactive protein in, 616
Serum precipitable iodine in, 874
Hexamethonium
Effect on arterial pressure, 435
Effect on blood flow in the foot, 786
Histamine
Of leukocytes, 679
Hydrazine Compounds
Effect on hypertension, 672
Hypersensitivity Reactions
Effect of albumin on, 660
Hypertension. See Blood, pressure, high
Hyperthyroidism. See Thyroid Gland, hyperthyroidism
Hyperuricemia
Effect of Benemid on, 889
Uric acid pool size and turnover rate, 879
Hypervolemia
Pulmonary hemodynamics in, 680
Hypoferremia, Acute
And relationship of the adrenal cortex to, 161
Hypopotassemia. See also Blood, serum, potassium; Electrolytes
Periodic paralysis without, 492
Hypoprothrombinemia. See also Blood, plasma
Effect of intravenous vitamin K_1 on, 680
Hypotension. See Blood, pressure, low

I

Immunization
In serum typhus, 656
Immunochemical Tests
Of tagged albumin, 1228
Immunology
Of trichinosis, 575
Inagglutinable Erythrocyte Counts, 1272
Infancy
Adrenocortical control of sodium and potassium, 318
Infection. See also Bacteria; Antibiotics
Effect on adenylic acid, 525
Hypoferremia in dogs and relationship of adrenal cortex, 161
Role in radiation syndrome, 651
Inflammation
Produced by chemical irritants, 1507
Influenza Virus
Inhibitor from sputum, 668
Vaccine, enhancement with adjuvant, 669
Inheritance
Of periodic paralysis, 492
Injury, Thermal
Anemia of, 181, 191
Insulin. See Pancreatic Preparations; Diabetes Mellitus
Effect on maximum capacity of renal tubules to reabsorb glucose, 125
Intracellular Water in Man, 1469
Iron
Ferrokinetics, 651, 1512
Irradiation
Damage to bone marrow, 667
Itch
Neural mechanisms in, 37

J

Jaundice, Obstructive
Serum precipitable iodine in, 874

K

Ketones in Whole Blood, 1483
Ketonuria
And steroid compounds, 1491
Fasting-induced, 1491
Khellin
Effect on cardiopulmonary function in chronic pulmonary disease, 1046
Kidney
Acute renal failure, pathogenesis, 1307
Adrenalin on renal function and electrolyte excretion, 1503
Afferent arteriolar resistance, calculation, 1143
Antidiuretic mechanism not regulated by extracellular fluid tonicity, 654
Artificial
Kinetics of hemodialysis, 683, 1062
Pressor effect of, 660
Binding, of mercurial diuretics, 1221
Clearance of radioiodine, 778
Cortical necrosis, in rabbits and hamsters caused by cortisone and bacterial toxins, 678
Degenerative glomerulonephritis, nocturnal diuresis, 642
Effect of toxins, trauma, ischemia and shock, 1307
Efferent arteriolar resistances, calculations, 1143
Electrolyte metabolism in nephritis, 421
Excretion
Adrenocortical control in newborn, 318
Of electrolytes influenced by chronic administration of adrenal medullary hormones, 908
Function
ACTH and cortisone effects in nephrosis, 658
ACTH-induced changes in nephrosis, 227, 661
Allantoin and creatinine clearances, relationship to filtration rate, 661
Creatinine clearance and protein consumption, 206
Effects of antidiuretic hormone on, 1055
Effect of Benemid on, 889
Following dialysis, 660
Following intravenous sodium bicarbonate, 674
Glomerular permeability and tubular recovery values for hemoglobin, 1041
Hemoglobin, effect on, 1033
In cirrhosis with ascites, 1200
In diabetes insipidus, 282
In severe osteomalacia, 679
Mercurial diuresis, effect on electrolytes, 1089
Nocturnal diuresis in heart and liver disease, 1191
Potassium excretion following acute renal injury, 1082
Potentiation of mercurial diuresis by ammonium chloride, 1105
Protoveratrine, action on, 659
Quiet standing, effect on glomerular filtration, 63
Renal function and aging, 457
Renal function in nephrotic syndrome, 471
Renal resistance, calculation of, 1143
Sodium depletion and repletion, effects on, 664
Urinary excretion of mercury, 1208, 1221
Urinary excretion of protein in normals, 853
Water diuresis produced by recumbency and intravenous isotonic saline, 862
Glomerular filtration rate, changes with quiet standing, 63
Hemodynamics after subtotal adrenalectomy, 633
Insufficiency. See also Uremia
Acute, 1307
Intracellular cation exchanges in acidosis due to, 381
Tubular excretion of potassium during recovery from, 1082
Intravenous digoxin in congestive failure, effects on, 1250
Ischemia, effect on dog kidney, 1328
Lower nephron nephrosis. See Kidney, insufficiency, acute
Mercurial diuretics, effects on urinary ammonia and titratable acidity, 1262
Nephrosis in children, effect of ACTH on, 471
Oxygen consumption in man, 745
Peritoneal lavage, effect on hemodynamics of dogs without kidneys, 644

Pyrogens, effects on renal hemodynamics, 457
Resistances, evaluation in normals and hypertensives, 1143
Response to sodium chloride, 1456
Role in intermediary metabolism, 634
Treatment of nephrosis
With ACTH, 1530
With cation resins, 1018
Tubules, effect of diabetes and insulin on glucose reabsorption by, 125
Kinetics
Iron in normal persons, 651, 1512
Of erythrocyte destruction in human beings, 1272

L

Larynx
Tuberculosis of, 445
Lead Poisoning, 1272
Leg
Effect of pressure on vascular volume of, 657
Leptospira Pomona Infection
Meningitis in, epidemiology of, 670
Leptospiremia
Duration after antibiotic therapy, 683
Leptospirosis
Treatment with antibiotics, 683
Leukemia. See also Blood
Amethopterin, citrovorum factor, and folic acid in, 631
Hemolytic factor in anemia of, 130
Mechanism of anemia in, 668, 1272
Monocytic and fat metabolism, effects of steroid compounds, 1491
Serum creatine tolerance in, 799
Studies on leukocytes of, 679
Uric acid pool and turnover rate in, 879
Leukopenia
Prevention following nitrogen mustard, 681
Lipids
In nephrosis, 637
Of serum in pregnancy, 388
Lipiodol
Effect on alveolar gas exchange, 338
Liver. See also Hepatitis
Blood flow
And bromsulphalein dosage, 658
Effect of upright posture in normals and hypertensive subjects on, 305
In hypertensives after sympathectomy, in various positions, 312
Capacity to produce antibody and effect of ACTH and cortisone, 647
Cirrhosis
Absorption studies from portal veins in, 263
Ascites, kidney function in, 1200
Blood flow in, 630
Electrolyte and fluid balance in, 1157
Hyponatremia in, 738
Nocturnal diuresis of water and sodium in, 642, 1191
Portal venous oxygen content in, 674
Renal effects of Pitressin in, 657, 663, 1287
Serum precipitable iodine in, 874
Sulfhydryl content of serum proteins in, 672
Turnover of albumin in, 1238
Disease
Effect of intravenous vitamin K_1 in, 680
Effect of Pitressin on the renal excretion of water and electrolytes in, 657, 663, 1287
Electrophoretic studies in, 718, 729
Hypergammaglobulinemia in young women with, 654
Serum precipitable iodine in, 874
Urinary excretion of choline metabolites in, 463
Function
After streptokinase-streptodornase, 1171
In burns, 191
Inactivation of estrogen *in vitro*, by liver slices, 678
Portal venous pressure, estimation of, 662
Lungs
Airflow, mechanics in health and in emphysema, 1175
Ambient air and residual volume determination, 639
Anoxia and human pulmonary vascular resistance, 957
Circulation
In health and disease, 680
Pulmonary pressures in pericardial tamponade, 661
Circulatory effects of raised airway pressure during cyclopropane anesthesia in man, 1243
Disease and hemodynamics, 680
Effect of intravenous dextran on pulmonary pressure, 897
Effect of lipiodol on alveolar gas exchange, 338
Effect of saline infusion on pulmonary arterial and pulmonary capillary pressure, 345
Emphysema
Cerebral circulation and metabolism in, 665
Mechanism of, 1175
Use of artificial respiration in, 838
Excretion, of radioactive carbon, 73
Function
Abnormal, use of respirator in emphysema, 838
Effect of khellin on, 1046
Effect of lipiodol on, 338
In health and emphysema, 1175
In sarcoidosis, 848
Hypertension, pulmonary, 680
Hypoxia as a respiratory stimulant, 648
Pulmonary arteriolar resistance and anoxia, 957
Pulmonary circulation and epinephrine, 707
Pulmonary pressure
Artery, 517
Capillary, 345
Measurement of, 635
Respiratory regulation during exercise in unconditioned subjects, 503
Sensitization of erythrocytes with tuberculin in pulmonary tuberculosis, 1542
Tuberculosis and ACTH, 445
Ventilation, 503
Virus pneumonia, 640
Lupus Erythematosus
Hemolytic anemia as presenting symptom in, 636
Lymphoma, Malignant. See also Leukemia
Mechanism of anemia in, 668

M

Magnesium
In plasma, 631
Malaria
Blackwater fever, pathogenesis of acute renal failure following, 1307
Media
Tween-albumin, 445
Meningitis
Leptospiral due to *Leptospira pomona*, 670
Polysaccharides of spinal fluid in, 582
Mental Status
Examinations, in pernicious anemia, 669
Mercurial Diuretics
Effect on edema in liver cirrhosis, 1157
Effect on renal ammonia and titratable acidity production, 1262
Excretion of Hg with, 1208
Hyponatremia from, 657
Mercuric Chloride
Pathogenesis of acute renal failure due to, 1307, 1314, 1337
Metabolic Rate, Basal
Relation to protein-bound iodine, 869
Metabolism. See also specific organ, tissue, substance
Calcium fructose diphosphate, 50
Comparison of effects of Compounds A, E, F and S acetate on, 665
Effect of ACTH on brain, 671, 1527

Effect of testosterone propionate on, 1486
Fasting-induced ketonuria, 1491
Fat, effect of steroid compounds on, 1491
Fat, in a patient with diabetes and thyrotoxicosis, 1486
Glucuronic acid, 685
Glycine, in rheumatoid arthritis, 655
Intermediary, role of kidney in, 634
Intracellular cation exchanges in alkalosis, 369
Of failing heart muscle, 630
Of radioactive carbon labelled acetate and glycine, 648
Serum albumin turnover rate, 1228
Serum albumin turnover rate in cirrhosis, 1238
Methods. See also Radioisotopes
ACTH, intravenous, test for adrenocortical function, 667
Adenine, estimation of total, 525
Adenosine diphosphate, estimation, 525
Adenosine triphosphate, estimation, 525
Antibodies, quantitative determination, 1451
Antibody nitrogen, precipitable, 1451
Arterial pressure, 435
Ashby counts, 1272
Bacteria artificially localized *in vivo*, 682
Ballistocardiograph and motion of thorax, 971
Bilirubin, "direct" and "indirect," crystallization, 663
Blood destruction *in vivo*, evaluation, 668, 1272
Blood flow and fluid compartments in skeletal muscle, measurement, 684
Blood fractionation with treponemal immobilizing antibodies, 659
Blood vessels in bulbar conjunctiva, examination of, 539
Blood volume
By carbon monoxide, 1441
With T-1824, 435, 575, 1441
Calcium and magnesium in plasma, determination of traces, 631
Calcium fructose diphosphate, metabolism, 50
Cardiac output
Continuous recording system for, 666
T-1824 method for, 897
Choline metabolites, determination of, 463
C-reactive protein, 616
Digital blood flow, 94
Diphenylamine reagent, and purple color reaction, 781
Electrophoresis
Of plasma proteins, 609
Of serum and plasma, 718, 729
Estrogens in presence of biological material, colorimetric determination of, 678
Extracellular fluid in man, sucrose for measurement, 1463
Flame photometer
Beckman, 1491
Perkin Elmer, 1503
Glucuronic acid, 685
Hemolysin titration, 11
Heparin tolerance, 948
Inagglutinable erythrocyte counts, 1272
Inflammation, quantitative determination of, 1507
Intracardiac and intravascular temperatures, 353
Intracellular water, antipyrine and sucrose for measurement of, 1469
Iodine, butanol-extractable, 531
Irradiation damage to bone marrow with radioiron, measurement, 667
Ketone bodies in biological fluids, colorimetric determination, 1483
Kidney function
And mercurial diuretics, 1262
In congestive failure after intravenous digoxin, 1250
Kidney resistances, calculation, 1143
Lipid mixtures, separation, 626
L-thyroxine, measurement of, 659
Middlebrook test in tuberculosis, 1542
Muscle cell membranes, transfer rates across, 684
Nephron dissection, mounting and staining, 1309
Non-endocrine factors responsible for composition of sweat, 325
Oximetric and pressure-recording techniques for cerebral circulation changes, continuous, 664
Oxygen
Availability with platinum electrodes, determination of, 670
Dissociation curve, *in vivo*, 666
Pitressin assay, 1287
Plasma, quantitative separation of small amounts, 99
Platelet extract, preparation, 77
Pleural pressure and rate of air flow, continuous record, 1175
Pneumococcus polysaccharide, 582
Portal venous pressure, estimation of, 662
Potassium accumulation in tissue slices, measurement, 662
Protein-bound iodine determination, 55
Protein components of small amounts of human plasma, determination, 99
Protein from electrophoretic cell, collection of, 853
Prothrombin, 596
Prothrombin consumption, 903
Pulmonary blood volume by T-1824, 345
Pulmonary capillary pressures, 957
Pulmonary excretion of radioactive carbon, 73
Pulmonary pressure, measurement, 635
Pyrogen test, standardized, 457
Rabbit ear chamber to observe connective tissue reactions, 636
Radioiodine
Extrarenal clearance of, 778
Protein-bound, determination, 1
Uptake and "accumulation gradient," correlation of, 301
Radioiron, vial counter for, 1512
Radioisotope, determination of body sodium, 584
Scintillation counters, 1512
Serum, labile factor, determination, 252
Sodium in body by isotopic dilution, 558, 566
Sulfhydryl content of serum proteins, determination of, 672
Sulfhydryl groups in serum, determination, 762
Thyroid hormone release by radioiodine, measurement of, 675
Thyroid metabolic rate, 659
Total body water, antipyrine for measurement of, 1469
Trypsin inhibitor on blood clotting, effect of, 1298
Turnover rate of tagged albumin, 1228
Ultracentrifugation, quantity, 674, 1071
Urinary osmotic loading with mannitol, 282
Urinary proteins by ultrafiltration, concentration of, 853
Visceral pain threshold in man, quantitative determination, 406
Middlebrook Test, 1542
Mineral. See substance, organ or tissue
Mineral Metabolism
In osteomalacia, 679
Mitral Stenosis. See Heart, mitral stenosis
Muscle
Cramps, following hypertonic glucose, 1111
Periodic paralysis without hypopotassemia, 492
Muscle, Skeletal
Blood flow, fluid compartments, transfer rates, 684
Mushroom Poisoning
Pathogenesis of acute renal failure following, 1307, 1317
Mutants
Use to reveal vitamins, 635
Myelofibrosis
Ferrokinetics, 1512

Myeloma, Multiple. See also Bence-Jones Protein; Blood
And hemolytic anemia, 1512
Relation of abnormal serum components to Bence-Jones protein, 669
Sulfhydryl content of serum proteins, 672
Myocardium. See Heart

N

Naphthoresorcinol
Light absorption curves, 685
Nephritis, Glomerular. See Glomerulonephritis
Nephrosclerosis
Role of somatotrophic hormone in, 673
Nephrosis. See also Kidney
Effect of ACTH and cortisone, 658
Electrolyte excretion with ACTH-induced diuresis, 661
In children, clinical, biochemical and renal hemodynamic features, 471
Mechanism of hyperlipemia, 637
Metabolism of cholesterol in, 657
Studies of sweat in, 680
Treatment with ACTH, 227, 1530
Neural Mechanisms
In itch, "itching skin" and tickle sensation, 37
Neurogenic Control
Evaluation in hypertension with tetraethylammonium chloride and spinal anesthesia, 925
Newborn
Adrenocortical control of sodium and potassium in, 318
Nitrogen Balance
During mercurial diuresis, 1089
Nitrogen Mustard
Prevention of leukopenia from, 681
Nor-epinephrine. See also Adrenal Preparations
Effect on pulmonary arteriolar resistance, 517
In urine, 641

O

Obesity
Protein-bound iodine in, 869
Obstetrical Complications
Pathogenesis of acute renal failure following, 1307
Oliguria
Following hemoglobin solutions, 1033
Osmotic Diuresis
Edema and hypertonic glucose, 1111
Of sodium and potassium in nephrosis, 661
Osteomalacia
Renal and metabolic changes in, 679
Otitis Media
Effect of cortisone on occurrence, 274
Oxygen
Consumption
In cirrhosis, 630
Of kidneys in man, 745
Effect in emphysema, 838
Intramyocardial availability, in acute coronary occlusion of dogs, 670, 932
Metabolism, of brain, following tilting in hypertension, 793
Therapy, in acute coronary occlusion of dogs, 670

P

Pain
Determination of visceral pain threshold, 406
Pancreas. See also Diabetes Mellitus
Testosterone propionate on metabolism in diabetes and thyrotoxicosis, 1486
Pancreatic Preparations
Insulin
Effect on maximum capacity of renal tubules to reabsorb glucose, 125
Labelled with radioiodine, 640
Panhypopituitarism. See Pituitary Gland, panhypopituitarism
Papaverine
Effect on cerebral circulation, 671
Para-aminobenzoic Acid
Effect on rickettsiae, 635
Paracentesis
Cause of hyponatremia in cirrhosis, 738
Parahemophilia, 627
Paritol
Effect on blood coagulation, 632
Paroxysmal Cold Hemoglobinuria
Hemolysis in, 11, 22
Observations on antibody, 22
Paroxysmal Tachycardia
Short P-R, normal QRS and, 658
Pellagrous Glossitis
Effect of adenylic acid and cozymase on, 628
Penicillin
Combined antimicrobial action on organisms resistant to, 631
Treatment of leptospirosis, 683
Penicillin Sensitivity
Effect of albumin on, 660
Peptidase Activity
Changes in blood with age, 84
Periarteritis Nodosa
Role of somatotrophic hormone in, 673
Pericardial Tamponade
Effect on pulmonary pressures, 661
Peritoneal Lavage
Effect on dogs without kidneys, 644
Pernicious Anemia. See also Blood
Mechanism of delirium in, 669
pH
Effect on blood coagulation, 629
Sodium bicarbonate, effect on, 674
Pheochromocytoma. See also Blood, pressure, high
Urinary excretion of epinephrine and nor-epinephrine in, 641
Phosphatase
Acid, of leukocytes, 679
Alkaline, of leukocytes, 679
Phosphoglucomutase
In heart and skeletal muscle, 671
Phosphorylase
In heart and skeletal muscle, 671
Pigment Excretion
In burns, 181
Pituitary
Acromegaly, ACTH on blood ketones in, 1491
Panhypopituitarism, effect of ACTH and cortisone on, 174
Role in response to anoxia, 638
Pituitary Preparations
Adrenocorticotrophic hormone
Action due to contaminant, 682
Analgesic effect of, 644
And cation exchange resins, 1009
And cortisone in thrombocytopenia, 638
And glucuronic acid, 685
And joint temperature, 701
Effectiveness of suspensions of, 665
Eosinophil response to, 629
In acute leukemia, 547
In adrenogenital syndrome associated with adrenal hyperplasia, 237
In chronic hepatic disease, 647
In hypertension, 630
In nephrosis, 658, 1530
In nephrotic syndrome, 227, 637
In panhypopituitarism and rheumatoid arthritis, 174
In tuberculosis, 445
Intravenous, 667
Loss of electrolytes after, 637
On body water and electrolyte distribution, 655
On body water, chloride and plasma volume, 684
On cerebral blood flow and metabolism, 671, 1527
On cholinesterase levels in ulcerative colitis, 662
On fasting-induced ketonuria, 1491
On migration of leukocytes, 659
On potassium metabolism, 627, 673
On serum lipids, 626
On sodium and potassium excretion in nephrosis, 661
On sodium and potassium excretion in newborn, 318
On sodium and water metabolism, 682

On sulfhydryl groups in disease, 762
On sulfhydryl metabolism in sickle cell anemia, 652
On uric acid pool and turnover rate, 879
Prevention of toxic effects of, 644
Relationship to acute hypoferremia, 161
"Resistance" to, 667
Anterior, effect on sodium and water metabolism, 682
Antidiuretic hormone
Decrease by recumbency and intravenous isotonic saline, 862
Effect on water and electrolyte excretion, 1055
Pitressin
Effect on cortisone-treated patients, 668
Effect on water and electrolyte excretion, 663, 1287
Induced water retention, effect on sodium excretion, 650
Posterior, relationship to adrenal cortex, 657
Somatotrophic hormone, production of disease by, 673
Thyrotropin, effect on release of thyroid hormone, 675
Plasma. See Blood, plasma
Plasma Acid-Base Volume
In mercurial and ammonium chloride diuresis, 1105
Plasma Albumin. See Blood, serum, albumin
Platelets. See Blood, platelets
Pneumococcal Capsular Polysaccharides
As antigens, 1451
Pneumonia Virus, 640
Poisoning
Pathogenesis of acute renal failure following, 1307, 1314
Polarograph
For determination of oxygen availability, 932
Polycythemia Vera. See also Blood
Ferrokinetics in, 651, 1512
Uric acid pool and turnover rate in, 879
Polysaccharides
In purulent meningitis, 582
Polyuria
Effect of osmotic loading in diabetes insipidus, 282
Porphyrin Excretion
In burns, 181
Portacaval Anastomosis, 630
Portal Pressure
Effect on bromsulphalein extraction, 630
Estimation, 662
Portal Veins
Anastomotic, absorption studies with, 263
Oxygen content of, 674
Posture
Effect on cerebral circulation, 664
Effect on hepatic blood flow, 305, 312
Tilting, effect on cerebral circulation in hypertension, 793
Potassium. See also Electrolytes; Blood, serum, potassium
Accumulation, in tissue slices, 662
Administration in hyponatremia, 675
Changes with ACTH, 627
Effect on hypertension, 639
Evidence for tubular excretion of, 1082
Metabolism, adrenocortical control in newborn, 318
Metabolism and excretion, following ACTH, cortisone and desoxycorticosterone acetate, 673
Osmotic loading and ACTH in nephrosis, 661
Retention in intracellular space in alkalosis, 369
Potassium Chlorate
Pathogenesis of acute renal failure following, 1307, 1316
P-R Interval. See also Electrocardiogram
Short, with normal QRS and paroxysmal tachycardia, 658
Pregnancy
High spinal anesthesia and renal hemodynamics in, 916
Precipitable iodine of serum in abortions and thyroidism, 137, 151
Precipitable iodine of serum in normal, 137
Serum lipids in, 388
Presidential Address, 43rd Annual Meeting of the American Society for Clinical Investigation, 623
Pressor Response
Of dialysis with artificial kidney, 660
Pressure
Effect on vascular volume of leg, 657
Procaine
Effect on cerebral circulation, 671
Procaine Amide
Clinical use, 676
Progesterone
In carcinoma of prostate, 547
Protein. See also Blood
Consumption of, and creatinine clearance, 206
Protein-Bound Iodine. See also Blood, serum, precipitable iodine
Radioactive, determination of, 1
Proteinuria. See also Urine, protein
Bence-Jones, and abnormal serum components, 1125
Prothrombin. See also Blood, plasma
Activity, effect of intravenous vitamin K_1, 680
Activity of human and dog blood, 903
Components for rapid conversion, 627
Consumption, relation of coagulase-reacting factor, 677
Conversion of, 596
Protoveratrine
Effect on circulation in hypertension, 651
Effect on kidney function, 659
In hypertension, 634
Pruritus
Neural mechanisms in, 37
Pseudohypoprothrombinemia, 627
Psychosomatic Medicine
Relationship of attitude and disease, 643
Pteroylglutamic Acid
Metabolism in scurvy, 639
Pulmonary. See Lungs
Pyridoxine
Deficiency and adrenal cortical function, 681
Pyrogen
Effect on renal hemodynamics, 457

Q

Quinidine
Study of failures following use for auricular fibrillation, 675
QRS Complex. See also Electrocardiogram
With short P-R interval and paroxysmal tachycardia, 658

R

Radiation Sickness
Effect of flavonoid compound, 395
Radiation Syndrome
Role of infection in, 651
Radioactive Glycine
Metabolism in man, 73
Radioactive Uric Acid
Measurement of pool size and turnover rate with, 879
Radioiodine
Bone marrow damage after, 667
Correlation of uptake and "accumulation gradient," 301
Tagged albumin
For cardiac output, 666
Turnover rate, 1228, 1238
Uptake in thyroid disease, 659
Radioiron
Measurement of hemoglobin synthesis with, 667
Radioisotopes
Carbon-labelled acetate and glycine in human beings, 648
Deuterium-labelled cholesterol, 657
Elimination of C^{14}, 630
Ferrokinetics of Fe^{59} *in vivo*, 651, 1512
I^{131} labelled insulin, 640

I^{131} labelled proteins in antibody response, 634
Na^{24} in congestive failure, 1471
P^{32} blood volume, 1471
Renal and extrarenal clearance of radioiodine, 778
Scintillation counters, 651, 1512
Renal. See Kidney
Reservoir
Size of uric acid, 879
Resins. See Carboxylic Cation Exchange Resins
Respiration. See also Lungs
Airflow in health and emphysema, 1175
Effect of CO_2, 503
Effect of O_2, 503
Ventilation, 503
Respiration, Artificial
In pulmonary emphysema, 838
Rheumatic Fever
C-reactive protein in, 616
Effect of cortisone on occurrence, 274
Purple color reaction of diphenylamine in, 781
Relation of serum antihyaluronidase titers in, 667
Rheumatism
Cortisone in, 628
Rheumatoid Arthritis. See Arthritis, Rheumatoid
Rickettsial Spotted Fever
Central nervous system sequelae as shown by electroencephalograms, 645

S

Saline Solution
Intravenous injection and pulmonary arterial pressure, 345
Sarcoidosis
Pulmonary function in, 848
Scurvy
Metabolism of folic acid and citrovorum factor in, 639
Sensations
Neural mechanisms in itch and tickle, 37
Serine
Kidney damage following, 1307, 1340
Serum Sickness
Alterations in blood volume and thiocyanate space, 360
Effect of albumin, 660
Shock
Effect on dog kidney, 1328
Hemorrhagic, physiology of, 683
Inadvisability of treating with hemoglobin solutions, 1033
Pathogenesis of acute renal failure following, 1307
Use of dextran in, 682
Shwartzman Phenomenon
Acquired resistance to, 628
Nature of blood vessel damage in, 676
Sickle Cell Trait. See also Blood
Abnormal hemoglobin, 1120
Skeletal Muscle
Biochemistry of, 671
Skin
Hypersensitivity to tuberculoprotein, 445
Neural mechanisms involved in "itchy skin," 37
Reactive hyperemia, in shock, 683
Sodium. See also Electrolytes
Change in body with age, 566
Chloride, renal response to intravenous injection, 1456
Depletion and repletion, effect on renal function and body fluids, 664
Depletion, in hypertension, 584
Estimation in body, 558, 566
Excretion
Effect of osmotic loading and ACTH in nephrosis, 661
Effect of Pitressin on, 657
Effect of Pitressin-induced water retention on, 650
Effect of quiet standing on, 63
Following glucose loading, 1111
Following intravenous sodium bicarbonate, 674
In mercurial and ammonium chloride diuresis, 1105
With mercurial diuresis, 1089
In infants, 566
In young adults, 558
Loss after ACTH and cortisone, 637
Metabolism
Adrenocortical control of, in newborn, 318
Effect of pituitary and adrenal hormones on, 682
Nocturnal diuresis in heart and liver disease, 642, 1191
Nitrite, effect on arterial pressure, 435
Restriction, dietary, in edema of liver cirrhosis, 1157
Space in congestive failure, 1471
Sodium Bicarbonate
Results of single intravenous dose in man, 674
Sodium Potassium Tartrate
Kidney damage following, 1307, 1337
Somatotrophic Hormone. See Pituitary Preparations
Sovasal 35, 1507
Spleen
Role in destruction of platelets in thrombocytopenia, 676
Sprue, Non-tropical
Effect of cortisone on, 678
Sputum
Fraction which restrains multiplication of influenza virus, 668
Standing
Antidiuresis of quiet standing, 63
Effect on soluble diuresis, 642
Starvation, Early
Diuresis in, 809
Steatorrhea, Idiopathic. See Sprue, Non-tropical
Stellectomy, Bilateral
Effect on cerebral circulation, 90
Steroids. See also Adrenal Preparations; Estrogens; Testosterone
17-keto, excretion following intravenous ACTH, 667
Urinary excretion, in adrenogenital syndrome with congenital adrenal hyperplasia, 237
Stomach
Adrenal phase of gastric secretion, 643
Streptococcal Infections
Antihyaluronidase response following, 667
Effect of cortisone on, 274, 678
Streptococci
Beta glucuronidase production by, 652
Streptodornase
Toxic responses to, 1171
Streptokinase
Toxic responses to, 1171
Streptomycin
Treatment of leptospirosis, 683
Stress
Emotional
Diuresis in, 809
Diuresis in diabetes mellitus during, 818
Relationship to acute hypoferremia, 161
Sucrose
Distribution between plasma and interstitial fluid, 1463
Sulfhydryl Groups
Associated with disease, 762
Effect on hypertension, 672
Metabolism of, in sickle cell anemia, 652
Of serum proteins in disease, 672
Sulfonamides
Pathogenesis of acute renal failure following, 1307, 1317
Swallowing Mechanisms
Studies of intraluminal esophagus pressures, 653
Sweat
As index of adrenal cortical activity, 325
Volume and composition, in normal children and nephrotics, 680
Sympathectomy
Cervical, effect on cerebral circulation, 90
Effect of tilting on cerebral circulation before and after, 793
Effect on hepatic blood flow in hypertensives in various positions, 312

Vasoconstriction following, 660
Sympathetic Nerve Tone
Effect on digital blood flow, 94
Syncope, Vasodepressor
Cerebral blood flow in, 664

T

Temperature
Comparison of intracardiac and intravascular with rectal, 353
Of joints in arthritis, 650, 701
Terramycin
Treatment of leptospirosis, 683
Testis
Effect of testosterone on, 648
Testosterone
Effect on metabolism, 1486, 1491
Effect on testis, 648
In prostatic cancer, 547
In rheumatoid arthritis, 633
Tetraethylammonium Chloride
Effect on digital blood flow, 94
In evaluation of neurogenic control of hypertension, 925
In hypertensives given ACTH and cortisone, 630
Thermal Injury
Liver function in, 191
Pigment excretion in, 181
Thiocyanate Space. See also Extracellular
In serum sickness, 360
In trichinosis, 575
Thorax
Motion during the heart cycle, 971
Thorotrast
To abolish tolerance to Shwartzman phenomenon, 628
Thrombin Formation
And trypsin inhibitor, 1298
Thrombocytopenia
Labile factor in serum, 252
Platelet survival in, 676
Thrombophlebitis
Clotting time and viscosity of blood in cases with, 672
Thromboplastin. See also Blood
Effect on hemophilic blood, 77
Effect on heparin tolerance, 948
Thyroid Gland. See also Blood, serum, precipitable iodine; Blood, serum, protein-bound iodine; Radioiodine
Butanol-extractable iodine of serum, 531
Correlation of uptakes and "accumulation gradient," 301
Effect of thyroid-stimulating hormone, 675
Feeding of desiccated thyroid to thyrotoxic subjects, 644
Hyperplasia, disproportionate to function of, 659
Hyperthyroidism
Adrenal cortical reserve in, 635
Nature of circulating thyroid hormone in, 1
Protein-bound iodine in, 55
Pulmonary hemodynamics in, 680
Serum creatine tolerance in, 799
Serum precipitable iodine in pregnancy with, 151
Metabolic rate, 659
Renal and extrarenal clearance of radioiodide in relation to function of, 778
Serum precipitable iodine in pregnancy, 137, 151
Testosterone propionate on metabolism in thyrotoxicosis, 1486
Thyrotoxicosis and fat metabolism, 1491
Tumors transplanted to anterior chamber of eye, 636
Thyroid Preparations
L-thyroxine, measurement, 659
Thyrotoxicosis. See Thyroid Gland
Thyrotropin. See Pituitary Preparations
Tonsillitis
Effect of cortisone on, 274
Toxemia of Pregnancy
Plasma proteins in, 609
Toxoid
Immunization in gas gangrene, 627
Transfusion
Pathogenesis of acute renal failure following, 1307
Transfusion Reactions
Effect of albumin in, 660
Trauma
Effect on dog kidney, 1331
Treponema Pallidum
Protein studies of immobilizing antibodies, 659
Trichinosis
Immunophysiology, 575
Triethylene Melamine
In Hodgkin's disease, 547
Trypsin
Effect of inhibitor on blood clotting, 1298
Relationship to blood coagulation, 112
Tuberculosis
Effects of ACTH and cortisone, 445
Erythrocytes sensitized with tuberculin in, 1542
Tuberculous Meningitis
Disturbances in electrolytes, 646
Tubular Resorption. See also Kidney, function
Of hemoglobin, 1041
Tumor. See also Cancer; Carcinoma
Malignant, adrenal cortex in, 632
Thyroidal, transplanted to anterior chamber of eye, 636
Turnover Rate
Of serum albumin, 1228, 1238
Of uric acid, 879
Typhus, Scrub
Immunization by combining living vaccine with chemoprophylaxis, 656

U

Ultracentrifuge
Analysis of tagged albumin, 1228
Quantity, distribution of serum proteins and lipids, 674, 1071
Uranyl Nitrate
Kidney damage following, 1307, 1338
Uremia. See also Kidney, function; Kidney, insufficiency
Effect of sodium depletion and repletion during, 664
Urethane
Effect on abnormal serum components and Bence-Jones protein, 1125
Uric Acid Metabolism
Effect of Benemid on, 889
Studies with radioactive uric acid, 879
Urine
Chloride, following stress in diabetes mellitus, 818
Electrophoretic patterns in Bence-Jones proteinuria, 1125
Excretion of choline metabolites, 463
Findings, after streptokinase-streptodornase, 1171
Glucose, following stress in diabetes mellitus, 818
Protein, amount and nature in normals, 853
Proteinuria after ACTH in nephrosis, 1530
Urobilinogen
In burns, 181

V

Vagus Nerve
Cardiac effects of reflex vagal stimulation, 656
Valvuloplasty
Effect on circulation in mitral stenosis, 643
Vascular Bed
Causes of constriction of, in digits, 660
Vena Cava
Effect of congestion of, on renal hemodynamics, 638
Venous Congestion of the Extremities, 1441
Venous Flow
Of extremities, effect of graded pressures on, 657
Venous Occlusion
Effect on digital blood flow, 94
Veratrum Viride
Effect on arterial pressure, 435
Virus Pneumonia
Mechanism of lung lesions, 640
Visceral Pain
Effect of inflammation and analgesics on, 406
Vital Capacity. See also Lungs
Effect of lipiodol on, 338

Vitamins. See also Pyridoxine
Action of vitamin B_{12} on human bone marrow, 31
Biological activity of a flavonoid compound, 395
K_1, intravenous administration in liver disease with hypoprothrombinemia, 680
"P," 395
P-hydroxybenzoic acid, 635
Thiamine, acute effects in beriberi heart disease, 654
Von Gierke's Disease
Differentiated from amyotonia congenita, 679

W

Water. See Body, water
Balance in cirrhosis of liver, 1157
Content of inflammation produced by chemical irritants, 1507
Content of skin, 1507
Diuresis, following cortisone, 668
Epidemic due to *Leptospira pomona* borne by, 670
Excretion
Diurnal variations in heart and liver disease, 642, 1191
During mercurial diuresis, 1089
Effect of Pitressin on, 657, 663, 1287
Effect of exogenous and endogenous antidiuretic hormone on, 1055
Quiet standing, effect on, 63
With increased plasma volume, 751
Metabolism
Effect of pituitary and adrenal hormones on, 682
Following hypertonic glucose in edematous patients, 1111
Retention, in cirrhosis, role of kidney, 1200
Wolff-Parkinson-White Syndrome
And syndrome of short P-R interval and normal QRS complex, 658

X

X-Ray Radiation
Survival of mice after exposure to, 629

Z

Zinc Protein
In leukocytes, 650

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CONTENTS

NUMBER 1, JANUARY, 1951

The Nature of the Circulating Thyroid Hormone in Graves' Disease. ISADORE N. ROSENBERG 1

The Mechanism of Hemolysis in Paroxysmal Cold Hemoglobinuria. I. The Role of Complement and its Components in the Donath-Landsteiner Reaction. WILLIAM S. JORDAN, JR., LOUIS PILLEMER, AND JOHN H. DINGLE 11

The Mechanism of Hemolysis in Paroxysmal Cold Hemoglobinuria. II. Observations on the Behavior and Nature of the Antibody. WILLIAM S. JORDAN, JR., LOUIS PILLEMER, AND JOHN H. DINGLE 22

Direct Action of Vitamin B_{12} upon Human Bone Marrow; the Effect of Instillations of Vitamin B_{12} and Folic Acid into the Bone Marrow as Studied by Nucleic Acid Staining Techniques. DANIEL HORRIGAN, THOMAS JARROLD, AND RICHARD W. VILTER 31

Neural Mechanisms Involved in Itch, "Itchy Skin," and Tickle Sensations. DAVID T. GRAHAM, HELEN GOODELL, AND HAROLD G. WOLFF 37

The Effect of Oral Administration of Calcium Fructose Diphosphate on the Serum Organic Phosphate, Inorganic Phosphate, Calcium, Protein, and Citric Acid Levels. SAMUEL NATELSON, MARGARET KLEIN, AND BENJAMIN KRAMER 50

The Clinical Determination of Protein-Bound Iodine. S. B. BARKER, M. J. HUMPHREY, AND M. H. SOLEY 55

Studies of the Antidiuresis of Quiet Standing: The Importance of Changes in Plasma Volume and Glomerular Filtration Rate. FRANKLIN H. EPSTEIN, ALLAN V. N. GOODYER, F. DOUGLAS LAWRASON, AND ARNOLD S. RELMAN 63

Studies in Glycine-2-C^{14} Metabolism in Man. I. The Pulmonary Excretion of $C^{14}O_2$. N. I. BERLIN, B. M. TOLBERT, AND J. H. LAWRENCE 73

Effect of Platelet Extract on Hemophilic Blood. ERNEST MOND AND KARL SINGER 77

Peptidase Activity in Leucocytes, Erythrocytes and Plasma of Young Adult and Senile Subjects. KARL STERN, MARION K. BIRMINGHAM, ANDREA CULLEN, AND RUTH RICHER ... 84

The Effect of Bilateral Stellectomy upon the Cerebral Circulation of Man. HENRY A. SHENKIN, FERNANDO CABIESES, AND GORDON VAN DEN NOORDT 90

The Effect of Venous and Arterial Occlusion, and Sympathetic Nerve Tone, on Digital Blood Flow. MILTON MENDLOWITZ, ARTHUR S. W. TOUROFF, AND HAROLD A. ABEL 94

Chemical, Clinical, and Immunological Studies on the Products of Human Plasma Fractionation. XL. Quantitative Separation and Determination of the Protein Components in Small Amounts of Normal Human Plasma. WALTER F. LEVER, FRANK R. N. GURD, EERO UROMA, RAY K. BROWN, BENJAMIN A. BARNES, KARL SCHMID, AND ETHEL L. SCHULTZ 99

Proteolytic Enzymes and Platelets in Relation to Blood Coagulation. BURTON L. TRAVIS AND JOHN H. FERGUSON 112

NUMBER 2, FEBRUARY, 1951

Effect of Diabetes and Insulin on the Maximum Capacity of the Renal Tubules to Reabsorb Glucose. SAUL J. FARBER, EUGENE Y. BERGER, AND DAVID P. EARLE 125

The Haemolytic Factor in the Anaemia of Lymphatic Leukaemia. G. MALCOLM-BROWN, S. MARGARET ELLIOTT, AND W. A. YOUNG 130

The Precipitable Iodine of Serum in Normal Pregnancy and its Relation to Abortions. EVELYN B. MAN, MARTIN HEINEMANN, CARL E. JOHNSON, DEBORAH C. LEARY, AND JOHN P. PETERS 137

The Precipitable Iodine of Serum in Pregnancy Complicated by Disorders of the Thyroid. WILLIAM W. ENGSTROM, DAVID M. KYDD, JOHN P. PETERS, AND EVELYN B. MAN..... 151
The Anemia of Infection. XIII. Studies on Experimentally Produced Acute Hypoferremia in Dogs and the Relationship of the Adrenal Cortex to Hypoferremia. G. E. CARTWRIGHT, L. D. HAMILTON, C. J. GUBLER, N. M. FELLOWS, HELEN ASHENBRUCKER, AND M. M. WINTROBE..... 161
Observations During ACTH and Cortisone Administration to a Patient with Longstanding Panhypopituitarism and Rheumatoid Arthritis. CHRISTIAN E. SCHROCK, RAYMOND F. SHEETS, AND WILLIAM B. BEAN..... 174
The Anemia of Thermal Injury. I. Studies of Pigment Excretion. G. WATSON JAMES, III, OLIVER J. PURNELL, AND EVERETT I. EVANS..... 181
The Anemia of Thermal Injury. II. Studies of Liver Function. G. WATSON JAMES, III, OLIVER J. PURNELL, AND EVERETT I. EVANS..... 191
Effect of Desoxycorticosterone Glucoside upon Cerebral Blood Flow and Metabolism of Human Subjects. R. C. BENTINCK, GILBERT S. GORDAN, JOHN E. ADAMS, L. H. ARNSTEIN, AND T. B. LEAKE..... 200
The Relation between Protein Consumption and Diurnal Variations of the Endogenous Creatinine Clearance in Normal Individuals. T. ADDIS, E. BARRETT, L. J. POO, H. J. UREEN, AND R. W. LIPPMAN..... 206
The Cardiac Output in Patients with Arteriovenous Fistulas. JAMES V. WARREN, JOHN L. NICKERSON, AND DANIEL C. ELKIN..... 210
The Effect of Temporary Occlusion of Arteriovenous Fistulas on Heart Rate, Stroke Volume, and Cardiac Output. JOHN L. NICKERSON, DANIEL C. ELKIN, AND JAMES V. WARREN. 215
The Blood Volume in Patients with Arteriovenous Fistulas. JAMES V. WARREN, DANIEL C. ELKIN, AND JOHN L. NICKERSON..... 220
The Effect of Adrenocorticotrophic Hormone on Children with the Nephrotic Syndrome. II. Physiologic Observations on Discrete Kidney Functions and Plasma Volume. HENRY L. BARNETT, CAROLYN W. FORMAN, HELEN MCNAMARA, WALLACE W. MCCRORY, MILTON RAPOPORT, ALEXANDER J. MICHIE, AND GUILIO BARBERO..... 227

NUMBER 3, MARCH, 1951

The Effects of Adrenocorticotropic Hormone and Cortisone in the Adrenogenital Syndrome Associated with Congenital Adrenal Hyperplasia: an Attempt to Explain and Correct its Disordered Hormonal Pattern. FREDERIC C. BARTTER, FULLER ALBRIGHT, ANNE P. FORBES, ALEXANDER LEAF, ELEANOR DEMPSEY, AND EVELYN CARROLL..... 237
The Labile Factor of Prothrombin Conversion: its Consumption under Normal and Pathological Conditions Affecting Blood Coagulation. BENJAMIN ALEXANDER, ROBERT GOLDSTEIN, AND GRETA LANDWEHR..... 252
Absorption Studies Using Portal Anastomotic Veins. WILLIAM BENNETT BEAN, MURRAY FRANKLIN, JAMES F. EMBICK, AND KATE DAUM..... 263
Effect of Cortisone on Acute Streptococcal Infections and Post-Streptococcal Complications. EDWARD O. HAHN, HAROLD B. HOUSER, CHARLES H. RAMMELKAMP, JR., FLOYD W. DENNY, AND LEWIS W. WANNAMAKER..... 274
The Mechanism of Polyuria of Diabetes Insipidus in Man. The Effect of Osmotic Loading. WILLIAM A. BRODSKY AND S. RAPOPORT..... 282
Factors Maintaining Cerebral Circulation During Gravitational Stress. JAMES P. HENRY, OTTO H. GAUER, SEYMOUR S. KETY, AND KURT KRAMER..... 292
Correlation of the 24-Hour Radioiodine Uptake of the Human Thyroid Gland with the Six- and Eight-Hour Uptakes and the "Accumulation Gradient." MONTE A. GREER..... 301

The Effect of the Upright Posture upon Hepatic Blood Flow in Normotensive and Hypertensive Subjects. JAMES W. CULBERTSON, ROBERT W. WILKINS, FRANZ J. INGELFINGER, AND STANLEY E. BRADLEY 305
The Effect of Splanchnic Sympathectomy in Hypertensive Patients upon Estimated Hepatic Blood Flow in the Upright as Contrasted with the Horizontal Position. ROBERT W. WILKINS, JAMES W. CULBERTSON, AND FRANZ J. INGELFINGER 312
Adrenocortical Control of Sodium and Potassium Excretion in the Newborn Period. ROBERT KLEIN 318
Studies on the Combined Use of Measurements of Sweat Electrolyte Composition and Rate of Sweating as an Index of Adrenal Cortical Activity. WILLIAM LOCKE, NATHAN B. TALBOT, HELEN S. JONES, AND JANE WORCESTER 325
The Effect of Lipiodol on Alveolar Gas Exchange. HARRISON BLACK AND ALBERT ROOS 338

NUMBER 4, APRIL, 1951

The Effect of Intravenous Infusions of Physiologic Saline Solution on the Pulmonary Arterial and Pulmonary Capillary Pressure in Man. JOSEPH T. DOYLE, JOSEPH S. WILSON, E. HARVEY ESTES, AND JAMES V. WARREN 345
Comparison of Intracardiac and Intravascular Temperatures with Rectal Temperatures in Man. LUDWIG W. EICHNA, ADOLPH R. BERGER, BERTHA RADER, AND WILLIAM H. BECKER 353
The Immunophysiology of Serum Sickness. Alterations in the Blood Volume and Thiocyanate Space in Relation to the Development of Humoral Antibodies in the Rabbit. JERRY K. AIKAWA AND GEORGE T. HARRELL 360
Intracellular Cation Exchanges in Metabolic Alkalosis. J. R. ELKINTON, R. D. SQUIRES, AND A. P. CROSLEY, JR 369
Intracellular Cation Exchanges in Acidosis Due to Renal Insufficiency. Effects of Alkali Therapy. J. R. ELKINTON, R. D. SQUIRES, AND R. B. SINGER 381
The Lipids of Serum in Pregnancy. JOHN P. PETERS, MARTIN HEINEMANN, AND EVELYN B. MAN 388
The Biological Activity of a Flavonoid (Vitamin "P") Compound. BORIS SOKOLOFF, WALTER H. EDDY, AND JAMES B. REDD 395
Cardiac Output in Postural Hypotension. J. B. HICKAM AND W. W. PRYOR 401
Quantitative Determination of the Visceral Pain Threshold in Man. EDWARD A. GAENSLER 406
Renal Function and Electrolyte Metabolism in Acute Glomerulonephritis. D. P. EARLE, S. J. FARBER, J. D. ALEXANDER, AND E. D. PELLEGRINO 421

NUMBER 5, MAY, 1951

The Collapse Produced by Venous Congestion of the Extremities or by Venesection following Certain Hypotensive Agents. EDWARD D. FREIS, JOSEPH R. STANTON, FRANK A. FINNERTY, JR., HAROLD W. SCHNAPER, ROBERT L. JOHNSON, CHARLES E. RATH, AND ROBERT W. WILKINS 435
Effects of Adrenocorticotropic Hormone and Cortisone in Patients with Tuberculosis. CHARLES A. LEMAISTRE, RALPH TOMPSETT, CARL MUSCHENHEIM, JAMES A. MOORE, AND WALSH MCDERMOTT 445
Aging as a Factor in the Renal Hemodynamic Changes Induced by a Standardized Pyrogen. ROGER K. MCDONALD, DAVID H. SOLOMON, AND NATHAN W. SHOCK 457
Urinary Excretion of Choline Metabolites following Choline Administration in Normals and Patients with Hepatobiliary Diseases. JESUS DE LA HUERGA AND HANS POPPER 463

The Nephrotic Syndrome in Children. An Interpretation of its Clinical, Biochemical, and Renal Hemodynamic Features as Variations of a Single Type of Nephron Disease. JACK METCOFF, W. M. KELSEY, AND C. A. JANEWAY 471
Studies in Disorders of Muscle. VII. Clinical Manifestations and Inheritance of a Type of Periodic Paralysis without Hypopotassemia. FRANK H. TYLER, F. E. STEPHENS, F. D. GUNN, AND G. T. PERKOFF 492
Respiratory Regulation during Exercise in Unconditioned Subjects. J. B. HICKAM, W. W. PRYOR, E. B. PAGE, AND R. J. ATWELL 503
The Effect of Nor-Epinephrine upon Pulmonary Arteriolar Resistance in Man. NOBLE O. FOWLER, RICHARD N. WESTCOTT, RALPH C. SCOTT, AND JOHNSON MCGUIRE 517
Blood Levels of Adenine Nucleotides. HARRY G. ALBAUM, THEODORE CAYLE, AND ARTHUR SHAPIRO 525
Butanol-Extractable Iodine of Serum. EVELYN B. MAN, DAVID M. KYDD, AND JOHN P. PETERS 531

NUMBER 6, JUNE, 1951

Peripheral Vascular Hemodynamics in the Bulbar Conjunctiva of Subjects with Hypertensive Vascular Disease. RICHARD E. LEE AND ELIZABETH A. HOLZE 539
Sensitivity of Serum Enzyme Inhibitors to a Variety of Cancer Chemotherapeutic Agents. FRANK W. ELLIS AND PHILIP M. WEST 547
Estimation of Total Body Sodium by Isotopic Dilution. I. Studies on Young Adults. GILBERT B. FORBES AND ANNE PERLEY 558
Estimation of Total Body Sodium by Isotopic Dilution. II. Studies on Infants and Children: An Example of a Constant Differential Growth Ratio. GILBERT B. FORBES AND ANNE PERLEY 566
The Immunophysiology of Trichinosis. Alterations in the Blood Volume and the Thiocyanate Space in Relation to the Development of Humoral Antibodies in the Rabbit. JERRY K. AIKAWA, GEORGE T. HARRELL, AND TREVA B. MILLER 575
Bacterial Capsular Polysaccharides in the Cerebrospinal Fluid of Patients with Purulent Meningitis. DANIEL L. LARSON 582
Dietary Treatment of Hypertension. II. Sodium Depletion as Related to the Therapeutic Effect. VINCENT P. DOLE, LEWIS K. DAHL, GEORGE C. COTZIAS, DOMINIC D. DZIEWIATKOWSKI, AND CECILIA HARRIS 584
Congenital SPCA Deficiency: A Hitherto Unrecognized Coagulation Defect with Hemorrhage Rectified by Serum and Serum Fractions. B. ALEXANDER, R. GOLDSTEIN, G. LANDWEHR, AND C. D. COOK 596
Plasma Proteins in Toxemias of Pregnancy. HAROLD C. MACK, ABNER R. ROBINSON, MARGARET E. WISEMAN, ERNEST J. SCHOEB, AND ICIE G. MACY 609
The Measurement of C-Reactive Protein in Human Sera. Comparison of the Clinical Tests on the Basis of a Quantitative Method. HARRISON F. WOOD AND MACLYN MCCARTY 616
Proceedings of the Forty-Third Annual Meeting of the American Society for Clinical Investigation held in Atlantic City, N. J., April 30, 1951 623

NUMBER 7, JULY, 1951

Investigation of Glucuronic Acid Metabolism in Human Subjects. W. H. FISHMAN, M. SMITH, D. B. THOMPSON, C. D. BONNER, S. C. KASDON, AND F. HOMBURGER 685
Effectiveness of a New Compound, Benemid, in Elevating Serum Penicillin Concentrations. JAMES M. BURNELL AND WILLIAM M. M. KIRBY 697

Joint Temperature Measurement in the Evaluation of Anti-Arthritic Agents. JOSEPH L. HOLLANDER, EMERY K. STONER, ERNEST M. BROWN, JR., AND PETER DEMOOR 701

The Effect of Epinephrine on the Pulmonary Circulation in Man. A. C. WITHAM AND J. W. FLEMING 707

Electrophoretic Studies in Liver Disease. I. Comparison of Serum and Plasma Electrophoretic Patterns in Liver Disease, with Special Reference to Fibrinogen and Gamma Globulin Patterns. MURRAY FRANKLIN, WILLIAM B. BEAN, WILLIAM D. PAUL, J. I. ROUTH, JESUS DE LA HUERGA, AND HANS POPPER 718

Electrophoretic Studies in Liver Disease. II. Gamma$_1$ Globulin in Chronic Liver Disease. MURRAY FRANKLIN, WILLIAM B. BEAN, WILLIAM D. PAUL, J. I. ROUTH, JESUS DE LA HUERGA, AND HANS POPPER 729

Hyponatremia in Hepatic Cirrhosis Following Paracentesis. WILLIAM P. NELSON, III, JACK D. ROSENBAUM, AND MAURICE B. STRAUSS 738

Studies of Renal Oxygen Consumption in Man. I. The Effect of Tubular Loading (PAH), Water Diuresis and Osmotic (Mannitol) Diuresis. JOHN KAPP CLARK AND HAROLD G. BARKER 745

The Effects of an Increase in Plasma Volume on the Metabolism and Excretion of Water and Electrolytes by Normal Subjects. L. G. WELT AND J. ORLOFF 751

The Determination of Sulfhydryl Groups in Serum. II. Protein Alterations Associated with Disease. EMANUEL B. SCHOENBACH, NORMAN WEISSMAN, AND ELEANOR B. ARMISTEAD. 762

An Estimation of the Renal and Extrarenal Clearance of Radioiodide in Man. WILLIAM M. MCCONAHEY, F. RAYMOND KEATING, JR., AND MARSCHELLE H. POWER 778

The Purple Color Reaction Given by Diphenylamine Reagent. I. With Normal and Rheumatic Fever Sera. WASHINGTON AYALA, LUCILE V. MOORE, AND EUGENE L. HESS 781

The Effect of Hexamethonium as Compared to Procaine or Metycaine Lumbar Block on the Blood Flow to the Foot of Normal Subjects. HAROLD W. SCHNAPER, ROBERT L. JOHNSON, EDWARD B. TUOHY, AND EDWARD D. FREIS 786

NUMBER 8, AUGUST, 1951

The Effects of 20° Head-up Tilt upon the Cerebral Circulation of Patients with Arterial Hypertension Before and After Sympathectomy. JOSEPH H. HAFKENSCHIEL, CHARLES W. CRUMPTON, HENRY A. SHENKIN, JOHN H. MOYER, HAROLD A. ZINTEL, HERBERT WENDEL, AND WILLIAM A. JEFFERS 793

Studies of Serum Creatine Tolerance. I. Findings in Normal Males and in Hyperthyroidism. JOHN H. PETERS, ROBERT SCHWARTZ, HOWARD MERMELSTEIN, MERCINE N. NEFORES, AND MATTHEW M. MANSUY 799

The Occurrence of Diuresis in Humans in Stressful Situations and its Possible Relation to the Diuresis of Early Starvation. LAWRENCE E. HINKLE, JR., CLIFFORD J. EDWARDS, AND STEWART WOLF 809

Studies in Diabetes Mellitus. II. The Occurrence of a Diuresis in Diabetic Persons Exposed to Stressful Life Situations, with Experimental Observations on its Relation to the Concentration of Glucose in Blood and Urine. LAWRENCE E. HINKLE, JR., CLIFFORD J. EDWARDS, AND STEWART WOLF 818

The Use of Artificial Respiration in Pulmonary Emphysema Accompanied by High Carbon Dioxide Levels. H. J. BOUTOURLINE-YOUNG AND J. L. WHITTENBERGER 838

Pulmonary Function Studies in Sarcoidosis. E. OSBORNE COATES AND JULIUS H. COMROE, JR. 848

The Amount and Nature of Urinary Proteins in Normal Human Subjects. DEMETRIOS A. RIGAS AND CARL G. HELLER 853

"Water Diuresis" Produced during Recumbency by the Intravenous Infusion of Isotonic Saline Solution. MAURICE B. STRAUSS, ROBERT K. DAVIS, JACK D. ROSENBAUM, AND ELSIE C. ROSSMEISL 862
Concentration of Serum Protein-Bound Iodine in Normal Men. ROBERT C. TUCKER AND ANCEL KEYS 869
Precipitable Iodine of Serum (SPI) in Disorders of the Liver. DAVID M. KYDD AND EVELYN B. MAN 874
Pool Size, Turnover Rate, and Rapidity of Equilibration of Injected Isotopic Uric Acid in Normal and Pathological Subjects. CHARLES BISHOP, WILLIAM GARNER, AND JOHN H. TALBOTT 879
The Effect of Benemid (p-[di-n-Propylsulfamyl]-Benzoic Acid) on Uric Acid Metabolism in One Normal and One Gouty Subject. CHARLES BISHOP, ROYDEN RAND, AND JOHN H. TALBOTT 889

NUMBER 9, SEPTEMBER, 1951

The Effect of the Intravenous Administration of Dextran on Cardiac Output and Other Circulatory Dynamics. A. C. WITHAM, J. W. FLEMING, AND W. L. BLOOM 897
On the Comparative Prothrombic Activity of Human and Dog Blood. ARMAND J. QUICK AND CLARA V. HUSSEY 903
The Effect of the Chronic Administration of Adrenal Medullary Hormones to Man on Adrenocortical Function and the Renal Excretion of Electrolytes. LEROY E. DUNCAN, JR., DAVID H. SOLOMON, MARY P. NICHOLS, AND ESTHER ROSENBERG 908
The Effect of High Spinal Anesthesia on the Renal Hemodynamics and the Excretion of Electrolytes during Osmotic Diuresis in the Hydropenic Normal Pregnant Woman. N. S. ASSALI, S. A. KAPLAN, S. J. FOMAN, R. A. DOUGLASS, AND Y. TADA 916
Evaluation of Neurogenic Control of Blood Pressure in Hypertension with Tetraethylammonium Chloride and Spinal Anesthesia. ALBERT A. BRUST, MORTON F. REISER, AND EUGENE B. FERRIS, JR. 925
Studies of Coronary Disease in the Experimental Animal. II. Polarographic Determinations of Local Oxygen Availability in the Dog's Left Ventricle during Coronary Occlusion and Pure Oxygen Breathing. J. J. SAŸEN, W. F. SHELDON, ORVILLE HORWITZ, PETER T. KUO, GEORGE PEIRCE, HARRY F. ZINSSER, AND JOHN MEAD, JR 932
Primary and Secondary Leucocyte Changes following the Intramuscular Injection of Epinephrine Hydrochloride. A. J. SAMUELS 941
The Effect of Intravenous Infusion of Thromboplastin on "Heparin Tolerance." ROBERT C. HARTMANN, C. LOCKARD CONLEY, AND JULIUS R. KREVANS 948
Anoxia and Human Pulmonary Vascular Resistance. RICHARD N. WESTCOTT, NOBLE O. FOWLER, RALPH C. SCOTT, VIRGIL D. HAUENSTEIN, AND JOHNSON MCGUIRE 957
The Motion of the Thorax during the Heart Cycle. A Comparison of Longitudinal, Lateral and Dorsoventral Ballistocardiograms. J. LEONARD BRANDT, ANTHONY CACCESE, WILLIAM DOCK, AND ALVIN SCHRAGER 971
An Introduction to Experimental and Clinical Studies of Carboxylic Cation Exchange Resins. T. S. DANOWSKI, L. GREENMAN, J. H. PETERS, AND F. M. MATEER 979
Carboxylic Cation Exchange Resin Effects in Dogs. T. S. DANOWSKI, L. GREENMAN, F. M. MATEER, W. B. PARSONS, F. A. WEIGAND, H. MERMELSTEIN, AND J. H. PETERS 984
Biochemical Changes Accompanying the Ingestion of a Carboxylic Cation Exchanger in the Hydrogen, Ammonium, Sodium, Potassium, or Calcium Form. L. GREENMAN, J. H. PETERS, F. M. MATEER, F. A. WEIGAND, D. WILKINS, R. TARAIL, G. RHODES, AND T. S. DANOWSKI 995

Acidifying and Non-acidifying Carboxylic Resin Mixtures Used Alone and with ACTH or Cortisone. J. H. Peters, T. S. Danowski, L. Greenman, F. A. Weigand, C. E. Clarke, K. Garver, F. M. Mateer, and R. Tarail 1009
Sodium Restriction and Cation Exchange Resin Therapy in Nephrotic Children. F. M. Mateer, Loraine H. Erhard, Marjorie Price, F. A. Weigand, J. H. Peters, T. S. Danowski, R. Tarail, and L. Greenman 1018
Probable Clinical Utility of Cation Exchange Resins. L. Greenman, J. H. Peters, F. M. Mateer, and T. S. Danowski 1027

Number 10, October, 1951

The Effect of Hemoglobin on Renal Function in the Human. John H. Miller and Roger K. McDonald 1033
Human Glomerular Permeability and Tubular Recovery Values for Hemoglobin. Roger K. McDonald, John H. Miller, and Eleanore B. Roach 1041
The Effect of Khellin on Cardio-Pulmonary Function in Chronic Pulmonary Disease. E. N. Silber, G. L. Snider, H. Goldberg, L. N. Katz, and D. B. Radner 1046
Effects of Exogenous and Endogenous Posterior Pituitary Antidiuretic Hormone on Water and Electrolyte Excretion. Richard J. F. Murphy and Eugene A. Stead, Jr. 1055
Artificial Kidney Function: Kinetics of Hemodialysis. A. V. Wolf, Donald G. Remp, John E. Kiley, and Gordon D. Currie 1062
The Study of Serum Proteins and Lipids with the Aid of the Quantity Ultracentrifuge. I. Procedure and Principal Features of the Centrifugate of Untreated Normal Serum as Determined by Quantitative Analysis of Samples from Ten Levels. Roy H. Turner, John Robert Snavely, William H. Goldwater, Malcolm L. Randolph, Charles C. Sprague, and Walter G. Unglaub 1071
Evidence Suggesting Renal Tubular Excretion of Potassium in Man During Recovery from Acute Renal Insufficiency. Jonas H. Sirota and Irving G. Kroop 1082
Electrolyte Equilibrium During Mercurial Diuresis. William B. Schwartz and William M. Wallace 1089
Potentiation of Diuretic Action of Mercuhydrin by Ammonium Chloride. James G. Hilton 1105
Effects of Injection of Hypertonic Glucose on Metabolism of Water and Electrolytes in Patients with Edema. Robert Tarail, Donald W. Seldin, and Allan V. N. Goodyer 1111
Familial Differences in the Proportion of Abnormal Hemoglobin Present in the Sickle Cell Trait. James V. Neel, Ibert C. Wells, and Harvey A. Itano 1120
Multiple Myeloma. IV. Abnormal Serum Components and Bence Jones Protein. R. Wayne Rundles, Gerald R. Cooper, and Robert W. Willett 1125
Evaluation of Renal Resistances, with Special Reference to Changes in Essential Hypertension. Domingo M. Gómez 1143

Number 11, November, 1951

Observations on the Alterations in Electrolytes and Fluid Balance in Patients with Cirrhosis of the Liver with and without Ascites. William E. Ricketts, Lillian Eichelberger, and Joseph B. Kirsner 1157
The Systemic Toxic Responses of Patients to Treatment with Streptokinase Streptodornase (SK-SD). William N. Hubbard, Jr. 1171
Mechanics of Airflow in Health and in Emphysema. Howard Dayman 1175
Studies in Diurnal Variation of Water and Electrolyte Excretion: Nocturnal Diuresis of Water and Sodium in Congestive Cardiac Failure and Cirrhosis of the Liver. Ralph Goldman 1191

Renal Function as a Factor in Fluid Retention in Patients with Cirrhosis of the Liver. Stephen H. Leslie, Barbara Johnston, and Elaine P. Ralli 1200
Urinary and Fecal Excretion of Mercury in Man following Administration of Mercurial Diuretics. J. Grossman, R. E. Weston, R. A. Lehman, J. P. Halperin, T. D. Ullmann, and L. Leiter 1208
Renal Extraction and Excretion of Mercury in Man following Intravenously Administered Mercurial Diuretics. R. E. Weston, J. Grossman, R. A. Lehman, T. D. Ullmann, J. P. Halperin, and L. Leiter 1221
The Turnover Rate of Serum Albumin in Man as Measured by I^{131}-Tagged Albumin. Kenneth Sterling 1228
Serum Albumin Turnover in Laennec's Cirrhosis as Measured by I^{131}-Tagged Albumin. Kenneth Sterling 1238
Circulatory Effects of Raised Airway Pressure during Cyclopropane Anesthesia in Man. Henry L. Price, Benton D. King, John D. Elder, Benjamin H. Libien, and Robert D. Dripps 1243
The Interrelationships of the Cardiovascular, Renal and Electrolyte Effects of Intravenous Digoxin in Congestive Heart Failure. Ludwig W. Eichna, Saul J. Farber, Adolph R. Berger, David P. Earle, Bertha Rader, Edmund Pellegrino, Roy E. Albert, J. Deaver Alexander, Harry Taube, and Sol Youngwirth 1250
The Effect of Mercurial Diuretics on Ammonia and Titratable Acidity Production in Acidotic Human Subjects with Reference to Site of Diuretic Action. Raymond E. Weston, Jacob Grossman, and Louis Leiter 1262
Studies with Inagglutinable Erythrocyte Counts. III. Kinetics of Erythrocyte Destruction in Human Beings. Raymond F. Sheets, Clinton D. Janney, Henry E. Hamilton, and Elmer L. DeGowin 1272
Studies in Edema. III. The Effect of Pitressin on the Renal Excretion of Water and Electrolytes in Patients with and without Liver Disease. Abraham G. White, George Rubin, and Louis Leiter 1287
The Site of Inhibition of Blood Clotting by Soy Bean Trypsin Inhibitor. Mary Beth Glendening and Ernest W. Page 1298

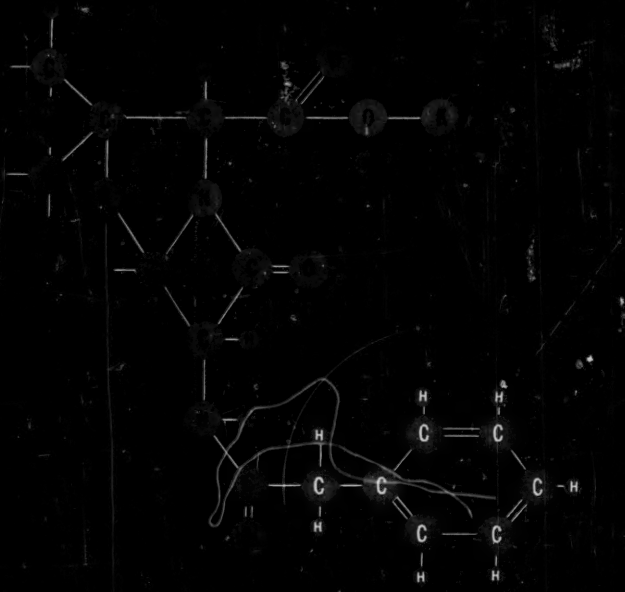
Number 12, Part I, December, 1951

The Pathogenesis of Acute Renal Failure Associated with Traumatic and Toxic Injury. Renal Ischemia, Nephrotoxic Damage and the Ischemuric Episode. Jean Oliver, Muriel MacDowell, and Ann Tracy 1305

Number 12, Part II, December, 1951

Venous Congestion of the Extremities in Relation to Blood Volume Determinations and to Mixing Curves of Carbon Monoxide and T-1824 in Normal Human Subjects. Ellen Brown, James Hopper, Jr., John J. Sampson, and Charles Mudrick 1441
Quantitative Antibody Studies in Man. I. The Effect of Adrenal Insufficiency and of Cortisone on the Level of Circulating Antibodies. Daniel L. Larson and Lois J. Tomlinson 1451
The Renal Response to Intravenous Injection of Sodium Chloride Solutions in Man. Betty Crawford and Henry Ludemann 1456
The Velocity of Distribution of Sucrose between Plasma and Interstitial Fluid, with Reference to the Use of Sucrose for the Measurement of Extracellular Fluid in Man. Norman Deane, George E. Schreiner, and James S. Robertson 1463
Intracellular Water in Man. Norman Deane 1469

Total Red Cell Volume, Plasma Volume and Sodium Space in Congestive Heart Failure. THEODORE C. PRENTICE, NATHANIEL I. BERLIN, GRACE M. HYDE, ROBERT J. PARSONS, JOHN H. LAWRENCE, AND SHIRLEY PORT 1471
Studies in Fat Metabolism. I. The Colorimetric Determination of Ketone Bodies in Biological Fluids. GEORGE D. MICHAELS, SHELDON MARGEN, GERALD LIEBERT, AND LAURANCE W. KINSELL 1483
Studies in Fat Metabolism. II. Evaluation of the Effect of Testosterone Propionate upon Ketone, Carbohydrate, and Protein Metabolism in a Patient with Diabetes Mellitus Complicated by Thyrotoxicosis. LAURANCE W. KINSELL, SHELDON MARGEN, GEORGE D. MICHAELS, AND DAVID P. MCCALLIE 1486
Studies in Fat Metabolism. III. The Effect of ACTH, of Cortisone, and of Other Steroid Compounds upon Fasting-Induced Hyperketonemia and Ketonuria. LAURANCE W. KINSELL, SHELDON MARGEN, GEORGE D. MICHAELS, ROBERT REISS, ROBERT FRANTZ, AND JOHN CARBONE 1491
The Effects of Adrenaline upon Renal Function and Electrolyte Excretion. W. E. JACOBSON, J. F. HAMMARSTEN, AND B. I. HELLER 1503
A Quantitative Relationship between the Water Content of Skin and Inflammation Produced by Chemical Irritants. H. VINIK WEISS, VINTON E. SILER, AND PETER R. BUECHLER 1507
Ferrokinetics in Normal Persons and in Patients Having Various Erythropoietic Disorders. REX L. HUFF, PAUL L. ELMLINGER, JOSEPH F. GARCIA, JOHN M. ODA, MARION C. COCKRELL, AND JOHN H. LAWRENCE 1512
The Effect of Adrenocorticotrophic Hormone (ACTH) on Cerebral Blood Flow and Metabolism. JAMES F. SCHIEVE, PERITZ SCHEINBERG, AND WILLIAM P. WILSON 1527
Treatment of Nephrosis with Pituitary Adrenocorticotrophin. JOHN A. LUETSCHER, JR., QUENTIN B. DEMING, AND BEN B. JOHNSON 1530
Hemagglutinins and Hemolysins for Erythrocytes Sensitized with Tuberculin in Pulmonary Tuberculosis. WENDELL H. HALL AND ROBERT E. MANION 1542
Index to Volume XXX 1547



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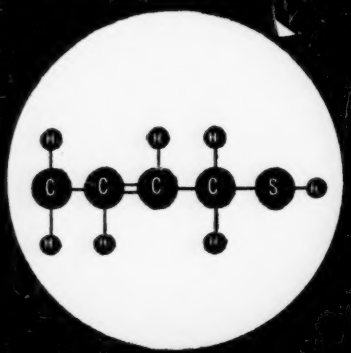

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Complete Epileptic Seizure-control; First Step in Rehabilitation

In the drug therapy of the epileptic, nothing short of *complete* suppression of seizures can rightly be called full success. Complete seizure-control is a prerequisite to the patient's return to a normally active life. Carter[1] states: "Basically all efforts should be directed toward preventing seizures and the tendency to chronic invalidism."

The management regimen should include:[1]

I. Good physical and mental hygiene.
   a) Wholesome diet; Regular bowel habits; Avoid noxious materials, e.g. alcoholics; Moderate physical activity.
   b) Help overcome feeling that epilepsy is shameful; Encourage normal work and recreation; Regular sleep without overprotection.

II. Anticonvulsant medication selected on the basis of Exact Diagnosis:
   "Certain drugs are more effective in one type of seizure than they are in another, and it is necessary to use proper drugs. The dosage must be individualized for each case."[2]

Thus, the best guide in selecting medication for a patient is the type of seizures present. (Frequently several drugs in combination is most effective.)

The table shows which drugs are indicated for each type of seizure. It will be noted that the newer hydantoin, Mesantoin, is effective for *grand mal*, Jacksonian and focal seizures.

MESANTOIN DOSAGE is adjusted to the lowest level adequate to attain freedom from seizures. Start with ½ or 1 tablet daily during the first week; increase the daily intake by ½ or 1 tablet during the next week. Continue this up to optimal dosage. When another drug has not given good results, Mesantoin is added by the same procedure; then the old drug is gradually reduced.

SIDE EFFECTS — Mesantoin produces side effects in some cases: Skin and blood changes are signs of sensitivity — the drug should be discontinued. Drowsiness develops upon reaching maximum dosage.

PRECAUTIONS — Certain precautions are necessary to minimize untoward effects.

a. close check on patient — repeated visits and blood counts.
b. No Mesantoin, if white cell count below 4000.
c. caution if (1) rash or blood changes.
   (2) History of drug sensitivity.
d. discontinue drug if (1) bleeding of gums or vagina.
   (2) Sore throat

TYPE OF EPILEPSY	PRIMARY CLINICAL MANIFESTATIONS	MEDICATION	AVERAGE ADULT DAILY DOSE★	LIMITING SIDE EFFECTS	SIGNS OF SENSITIVITY
GRAND MAL	Aura followed by loss of consciousness; tonic-clonic convulsions and autonomic disturbance, e.g., in bladder function.	Phenobarbital	1¾ grs. h.s. Usual max.: 3 grs. per day	Sleepiness	
		5,5 diphenyl hydantoin	1¾ grs. t.i.d., p.c. Usual max. 6 grs. per day	Diplopia, staggering (may increase petit mal)	Overgrowth of gums, body hairs; itching rash on extremities, gastric irritation
JACKSONIAN	Convulsions begin in one area and spread outwards (Jacksonian march.). No loss of consciousness.[3]	3 methyl 5,5 phenylethyl hydantoin (mesantoin)	Adults: 2 to 6 tabs. Children: 1 to 4 tabs.	Sleepiness	For full details see Psychomotor cross-column below
		phenylacetylurea[5]	2 Gms. per day. Max. dose 6 Gms. per day[5]	Drowsiness[5]	Central nervous system affected, nausea, vomiting and abdominal pain[5]
PSYCHOMOTOR	Period of amnesia; actions apparently purposeful but mechanical; incoordination. Mild tonic spasms. In children: "behavior problems".[5]	3 methyl 5,5 phenylethyl hydantoin[4] (mesantoin)	Adults: 2-6 tabs. Children: 1-4 tabs.	Pharyngitis, mucous membrane bleeding, lymphadenopathy, measles-like rash with itching and fever; in severe reaction: blood dyscrasia.	
		phenylacetylurea[5]	See phenylacetylurea—above		
PETIT MAL TRIAD	Attacks: in series (rapid succession), abrupt onset, few sec. duration; no aura nor impairment of consciousness. Rhythmic twitching (e.g. eyelids).	3,5,5 trimethyl oxazolidine 2,4 dione	0.9 to 1.2 Gms.	Inability to see in a bright light. Drowsiness	Hiccough, acneform rash, leukopenia
		N methyl derivative of phenobarbital	6 grs.	Sleepiness	

★Although an average daily dose can be stated for each of these drugs, to obtain best results it is necessary that the physician determine the dose required by each individual patient for maximum control of his seizures.

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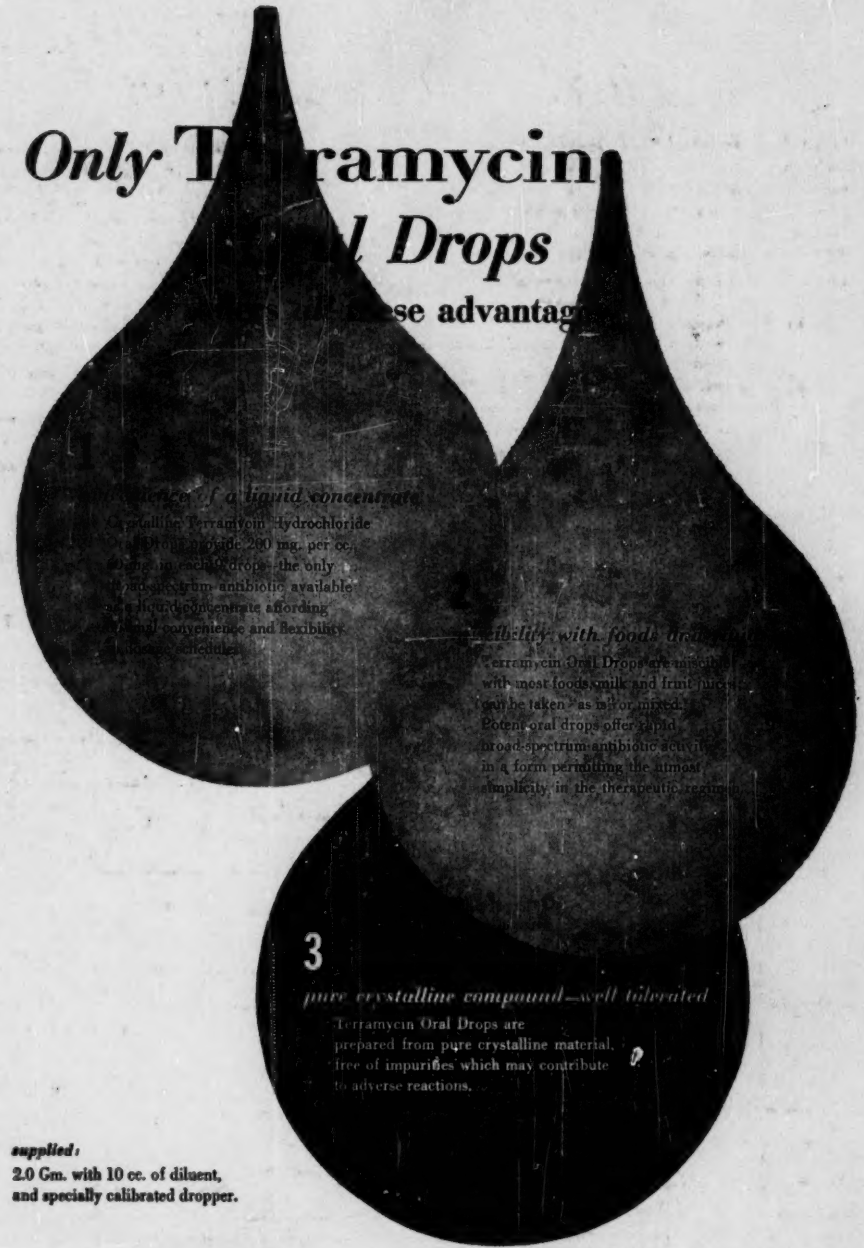

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